THE EARLY DEVELOPMENT OF DINOPHILUS: A STUDY IN CELL-LINEAGE.

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CONTENTS.

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I.—INTRODUCTORY AND HISTORICAL.

The phylogenetic relationships of *Dinophilus* have presented a perplexing problem to morphologists since the establishment of the genus by Oscar Schmidt in 1848. The various views as to its systematic position may be conveniently classed under three heads: (1) those referring *Dinophilus* to the Turbella ia; (2) those referring it to the

Nemertina, and (3) those referring it to the Annelida, or to some position intermediate between the annelids and the rotifers. The older writers, with the exception of van Beneden, referred the genus to the Turbellaria, on account of its obvious external resemblances to that group. Among these authors may be mentioned Schmidt (1848) (who, however, later changed his opinion), Max Schultze (1849), Diesing (1874), Mereschkowsky (1879), Korschelt, in his first paper on *Dinophilus* (1882), and Weldon (1886).

In 1861 van Beneden described a species from the coast at Ostende, and referred the genus to the nemerteans, principally, it would seem, on account of the character afforded in the possession of a proboscis. Of recent writers who have inclined to this opinion we have only Verrill (1895), who, however, does not enter into a discussion of the relationships of the group, but only provisionally refers it to the nemerteans.

The first to place *Dinophilus* among the annelids was Schmarda (1861), who described a species from the coast of South America, and assigned it to a place in the Oligochæte family of the Naidæ, next to the primitive genus *Ælosoma*. The claims of *Dinophilus* to a place among the annelids have, however, been based chiefly on its remarkable resemblance to certain annelid larvæ, especially those of the polytrochal type. The first to call attention to this fact was Metschnikoff (1866), who in his paper on *Apsilus* wrote concerning the systematic position of *Dinophilus:* "Dass Dinophilus als eine stationäre Anneliden-larva zu betracten ist, und mithin zu der Anneliden ebenso wie Appendicularia zu den Ascidien sich verhält." He also notices some resemblances to certain rotifers. As a curious parallel to this view may be cited Oscar Schmidt's (1882) comparison of the position of *Dinophilus* among the annelids to that of *Axolotl* among the true salamanders.

Graff (1882), in his fine monograph on the Turbellaria, removes *Dinophilus* from the Turbellaria, and considers it as more properly belonging near the Annelida.

Lang (1884) places *Dinophilus* in the line which leads through the Archiannelida to the rotifers. Harmer (1889) also regards *Dinophilus* as nearly related to the Archiannelida; Repiachoff (1886) considers it a true annelid, as does Korschelt (1893); while of the most recent writers Schimkewitsch (1895) considers *Dinophilus* as affording characters which relate it to both the rotifers and annelids.

These various views have been based almost entirely on anatomical evidence, since but three papers deal with the embryology, viz.: Korschelt (1882), Repiachoff (1886) and Schmikewitsch (1895).

Korschelt studied the living egg and described the manner in which the eggs were laid, the early stages of the cleavage, an epibolic gastrulation, and observed that at hatching the young *Dinophilus* closely resembles the adult. Repiachoff's paper is more complete as regards the embryology, giving numerous figures of the cleavage and sections of the gastrula. Two important discoveries are to be attributed to this investigator, viz.: That the mesoderm arises from a pair of mesoblast cells situated posterior to the blastopore, and that a considerable portion of the ectoderm of the adult arises from a pair of large cells situated at the posterior end of the embryo and derived from the largest cell of the 4-cell stage. Schimkewitsch follows along the same lines as Repiachoff, and does little more than confirm the latter's results, though the figures given by Schimkewitsch are much in advance of those given by Repiachoff. These authors, Korschelt, Repiachoff and Schimkewitsch, attempted to compare the cleavage of the *Dinophilus* ovum with that of the rotifers and consequently failed to properly interpret it.

In view of the fact that so little is at present known concerning the early development of this form, the evidence as to its relationships being principally anatomical, it has seemed highly desirable to study the embryology thoroughly, from the earliest cleavage on.

The present paper is concerned almost wholly with the cell-lineage. This has been done for two reasons: first, because such a careful study of the cleavage as is involved in a study of the cell-lineage gives a firm and secure basis for work on the later development; and second, because the study of the cell-lineage of mollusks and annelids has brought to light such striking resemblances that there can scarcely be any doubt that they are of phylogenetic significance.

II.—Material and Methods.

The species of *Dinophilus* with which this paper is concerned has not been determined with certainty. Both sexes correspond closely to the description given by Korschelt (1882) for the species found by him in aquaria at Freiburg, and named by him *Dinophilus apatris*. Repiachoff (1886) has supposed that this species is identical with *D. gyrociliatus* O. Schmidt. The individuals of the species found in the aquaria at the University of Pennsylvania agree with *D. apatris*, and differ from *D. gyrociliatus* in lacking the last or perianal circle of cilia, and also appear to differ from *D. gyrociliatus* in another important respect, *i.e.*, in having no segmental organs. E. Meyer (1887) figured for the females of *D. gyrociliatus* five pairs of nephridia of the type found

in annelid larvæ (protonephridia). Careful study of fixed and living material has thus far afforded me no evidence whatever of nephridia of any sort.

The ova which were studied in the preparation of this paper were collected from the sea-water aquaria in the vivarium of the University of Pennsylvania during the months of January to May, 1902.

The ova are laid in gelatinous capsules as described by Korschelt for *D. apatris*, each capsule containing three to seven ova of two sizes, the smaller about one-third the diameter of the larger. The smaller ova give rise to the minute and degenerate males, while the larger ova give rise to the female individuals. The number of the large female ova exceeds that of the male. In fifty capsules 214 ova were counted, of which number 79 were male ova and 135 female. These latter have been the object of my investigation, the small size (ca. 30 micra in diameter) and smaller number of the male ova rendering them much less favorable for study. The capsules were found attached to the various sea-weeds in the aquaria, and particularly to the *Ulva*. A quantity of the sea-weed was taken from the tanks in which the animals were found to be most abundant and squeezed over a large watch crystal. The eggs thus washed out from their capsules, together with much vegetable débris, soon settled to the bottom of the watch crystal, from which they were picked out, under a lens, by means of a fine-pointed pipette and transferred to a small vessel. The vessel found most useful for this purpose was made from the hemispherical bottom of a small test-tube cemented to a slide. The ova collected in this manner proved to be in all stages of development, from the unsegmented ovum to an embryo ready to escape.

The ova were in all cases killed with Kleinenberg's stronger picro-sulphuric fluid, and after washing in 70 per cent. alcohol, were stained with Conklin's (1902) picrohæmatoxylin. This method of fixing and staining has proved satisfactory with so many forms that it was considered advisable, in view of the scarcity of the material, not to experiment further. The ova were then dehydrated, cleared in cedar oil or xylol, and mounted in balsam under covers supported by thin glass feet, thus providing a space in which the eggs may be rolled about by displacing the cover glass.

It has been noted by several observers that *Dinophilus* practically disappears at the approach of warm weather, and this fact was found to be true in this case also. This is interpreted by Korschelt to mean simply that the period of sexual activity has come to an end. However, species of *Dinophilus* have been found at Wood's Hole, Massachu-

setts, during the summer months by Verrill (1895) and by Miss Moore (1899), so that it would appear that some few individuals lived over the summer.

The animals found in the aquaria were probably imported on sea-weed gathered at Wood's Hole, Massachusetts, or at Sea Isle, N. J., and owing to the favorable conditions afforded by the aquaria they multiplied and became abundant During the past three seasons, however, *Dinophilus* has not become abundant until late in the autumn or in the early winter. The largest number of ova were gathered in January and February, the number diminishing from that time, until by May their number was so small that it did not pay to collect them. During the past three years the animals have nearly all disappeared by the middle of June.

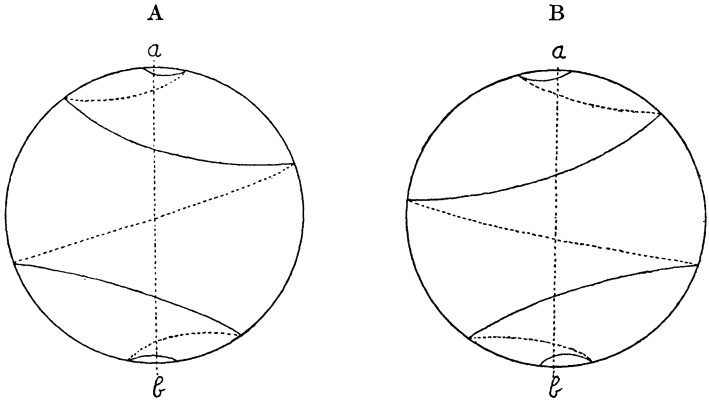


Fig. I. A and B, diagrams of ova with circumscribed loxodromic curves. a–b, egg axis. Copied from Mead (1897).

III.—NOMENCLATURE.

The nomenclature adopted in this paper is that modification of Wilson's system (1892) used by Conklin (1897), with the further modification adopted by Child (1900) in prefixing coefficients to the macromeres, as well as to the micromeres. The macromeres are designated by capitals, the micromeres by small letters.

When the cleavage plane of two cells approximates the direction of the loxodromic curve shown in text fig. I, A, it is dexiotropic; when it approximates the direction of that shown in text fig. I, B, it is leiotropic.

The number of the quartette is indicated by a coefficient. Thus 1a is the member of the first quartette located in the A quadrant. The

product of a division which lies toward the animal pole receives the exponent 1, that toward the vegetal pole the exponent 2. Thus $1a^1$ lies nearer the animal pole than $1a^2$. If the cleavage is meridional the right cell, as seen by an imaginary observer located at the animal pole, receives the larger exponent. Thus $1a^{1.1}$ lies to the right of $1a^{1.2}$.

The macromeres receive a coefficient corresponding to the number of the quartette to which they last contributed. Thus 4A last gave rise to 4a.

When cells arise whose origin and fate are similar to those of annelids or mollusks which have already received special names, as, for example, the "trochoblasts" or the "intermediate girdle cells," I have made use of these names. I do not, however, wish to imply that the cells are necessarily homologous with those to which these names were first applied.

The animal pole is that point at which the polar bodies are given off, the point opposite is the vegetal pole.

IV.—History of the Cleavage.

(1) *Unsegmented Ovum.*

My observations on the unsegmented ovum have unfortunately been confined entirely to fixed and stained material. Since in each lot of material all stages were found, from the unsegmented ovum to the larva about to hatch, a considerable amount of time and labor would have been involved and many ova lost in selecting for study the few which were still in an unsegmented condition. The ova are approximately spherical and not elongated in one dimension, as Korschelt describes them in *D. apatris*. Measurements of the diameters of six unsegmented eggs were respectively 108 micra, 100 micra, 90 micra, 96 micra, 92 micra and 100 micra, giving as the average diameter of the egg 97.66 micra. These measurements nearly approach those given by Korschelt for *D. apatris, i.e.*, 111 micra x 92 micra.

Closely surrounding the ovum is a delicate wrinkled vitelline membrane. The protoplasm in the living ovum is nearly opaque, this opacity being due to the presence of minute deutoplasmic spheres uniformly distributed throughout the cytoplasm. These deutoplasmic spheres give to the stained and mounted ova a darkly granular appearance, which in many cases makes both mitotic figures and cell outlines difficult to distinguish. Fig. 1[1] shows the ovum just after the extrusion of the second polar body. The latter is spherical in shape and about half as large as the first polar body, which is somewhat ovoid. In the first polar body a faint nucleus can be made out, but none in the

[1] See Plates XLIII–XLVIII.

second. Neither of them have been seen to divide. The polar bodies in *Dinophilus*, as in all animals, mark the animal pole, but are, however, not a reliable means of orienting the later stages, since through some cause they tend to become displaced and are ultimately taken into the cells over which they happen to lie. In fig. 8, for example, the first polar body is already sinking into the cell 1c, while the second is still free.

In fig. 1, beneath the polar bodies lies the female pronucleus, formed of four nuclear vesicles, each vesicle resembling a small nucleus in appearance, having a distinct bounding membrane and containing small granules of chromatin of varying sizes. Below the vesicles, and containing them as in a cup, is a large hemispherical aster. Somewhat below and to the right of the center of the ovum lies the male pronucleus with its accompanying aster, which lies on the vegetal pole side of the nucleus.

The stage next studied is represented in fig. 2, where the two pronuclei are seen to have come together. The male pronucleus is probably the large bilobed vesicle which lies on the side of the nucleus toward the vegetal pole; the female pronucleus is probably represented by the eight smaller vesicles on the other side. The ovum is in the early prophase of division, a large and deeply staining aster being on each side of the nucleus, though no spindle fibres can yet be distinguished.

(2) *Primary Cleavages.* 1–4 *Cells.*

The spindle for the first cleavage is shown in fig. 3. The cell body has elongated and the spindle is in the anaphase, while the unequal character of this cleavage is clearly indicated by the inequality in the diameter of the two asters as well as by the eccentric position of the spindle. The spindle is inclined at a slight angle to the horizontal plane, the end which is to form the smaller cell being the lower. The explanation of this is not clear; possibly the spindle was oscillating, or perhaps the position of the spindle may have something to do with the beginning of spiral cleavage. Unfortunately this is the only ovum which I have seen in which the spindle is in either metaphase or anaphase, so that it is not absolutely certain that this oblique position is normal. The plane of this division passes through the animal pole, but lies to one side of the pole opposite. The products of this division (fig. 4) are very unequal, the cell C–D greatly exceeding A–B in size.

Immediately after division both cells prepare to divide again, but the two halves of the second cleavage are not simultaneous, C–D divid-

ing much in advance of A–B, as fig. 5 shows. As seen in the figure, the spindle in C–D has reached the late anaphase, the chromosomes lying close to the centrosomes, while a double row of microsomes has appeared on the spindle fibres. On the other hand, in A–B the spindle has only just reached the metaphase. The division of A–B is nearly equal, A being slightly the larger product, while the division of C–D is highly unequal. The spindles for both divisions are inclined in a leiotropic direction, as is especially well shown by the division of A–B in fig. 6, in which the left pole of the spindle is much higher than the right.

The four cells formed by the second cleavage all differ in size (figs. 6 and 7, text fig. II, A and B); while D is relatively colossal, C, B, and A are more nearly alike. Of these C is the largest, A slightly smaller than C, while B is the smallest of all. The enormous size of D can be

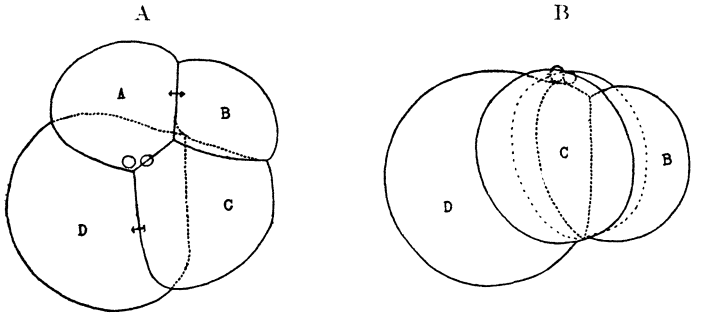


Fig. II. A, 4-cell stage from animal pole; B. same from right side.

appreciated only when the 4-cell stage is viewed from the side, as shown in text fig. II, B.

The size relation of the four cells A, B, C and D appears to be somewhat unusual. In all Annelida and Mollusca investigated which have unequal cleavage the left posterior blastomere D is the largest one of the 4-cell stage, except in the case of *Aplysia* (Blochmann, 1883; Carazzi, 1900) and the *Pteropoda thecosomata* (Fol, 1875). A and B are usually equal, while C is more or less intermediate between B. and D. Among the Lamellibranchia there appear to exist cases parallel to that of *Dinophilus*. An examination of the figures given for *Cyclas* (Stauffacher, 1893), for *Unio* (Lillie, 1895) and for *Dreissensia* (Meisenheimer, 1901) seems to indicate that in these forms B is the smallest of the four cells, while it is fairly evident that C exceeds A in size, although I find no precise statement of these facts in the text of the papers mentioned above. In all these forms D is relatively enormous.

In the 4-cell stage of the *Dinophilus* ovum but one polar furrow is usually present, that at the animal pole, formed by the junction of A and C. This furrow is very long and turns to the right when seen in the second cleavage plane. At the vegetal pole all four cells meet at a point (figs. 6 and 7, text fig. II, A and B). The condition where a polar furrow exists at the animal pole is also found in the Lamellibranchs mentioned alone; in these, however, the furrow is formed by the junction of B and D. The phenomenon of a long polar furrow existing at the animal pole in *Unio* is explained by Lillie (1895) as being due to the fact that "the greater mass of the first blastomeres is ectodermal." This explanation fits the case of *Unio* very well, since the blastomere D which contains the greatest mass of ectoderm takes part in the formation of the polar furrow, but does not explain the situation in *Dinophilus*, where D is entirely excluded from the polar furrow. This condition is probably due to two factors: (1) the extreme obliquity of the second cleavage spindles; and (2) to the relatively small size of B. The inclination of the spindles in A–B and C–D cause A and C to lie above B and D and so meet in a long cross furrow. Were B as large as either A or C it would meet D in a furrow at the vegetal pole, but since its mass is so much less than that of the other cells it just touches D at the vegetal pole.

The somewhat complex relation of the first and second cleavage planes to the embryonic axis will be discussed in a later section; for the purpose of convenience in description, however, the blastomeres A and B will be considered as anterior, C and D as posterior.

(3) *Segregation of Ectoblast.* 4–26 *cells.*

Immediately after the second cleavage of the ovum the nuclei of the blastomeres resume mitotic activity. In fig. 6 asters are seen in all four cells, while between A and C there are still the remains of spindle fibres to be seen. In D the asters have assumed nearly their definitive position, indicating where the ends of the dexiotropic spindle are to lie.

At this time there comes to light a curious and striking feature of the cleavage of the *Dinophilus* egg, mentioned by Lang (1884) as occurring in *Discocœlis*, by Lillie for *Unio* (1895) and by Jennings for *Asplanchna* (1896), also by Child (1900) as occurring occasionally in *Arenicola* at the third cleavage. This peculiarity consists in the fact that the macromeres never divide simultaneously, but always successively and in a regular and invariable order. This order is the same as that of *Unio* and *Arenicola*, *i.e.*, D, C, A, B, while that of *Asplanchna*

is D, C, B, A. This order of division is maintained by the macromeres through five successive cleavages, and is more or less perfectly retained by their descendants. This succession is well shown in the ovum represented in fig. 8, where the nuclei of the cells 1D, 1C, 1d and 1c are in the prophase of the next division, while a bundle of spindle fibres still connects the nuclei of 1A and 1a and the micromere 1b is scarcely yet cut off from 1B. This sequence in the division of the macromeres is clearly correlated with the difference in the sizes of A B, C and D, since it has already been pointed out that in relative size the macromeres follow one another in the order D, C, A, B, which is also the order of their division. It is evident that this is only a special instance of a widespread class of phenomena, discussed by Kofoid (1894), who points out that in many ova the larger blastomeres, *i.e.*, those containing a greater amount of yolk, or rather more cytoplasm, tend to divide more rapidly than do the smaller ones. This, as Kofoid shows, is in contradiction to Balfour (1880), who formulated a law which supposes yolk to retard the cleavage. Kofoid ingeniously explains the contradiction by suggesting that "difference in the rapidity of cleavage is apparently correlated with the greater or less absolute amount of protoplasm," and that the amount of protoplasm in turn may be increased through the appropriation of yolk and the ratio of division may be thus indirectly hastened. In *Dinophilus*, however, while it seems tolerably plain that the difference in the time of division of the macromeres is related to their difference in size, yet the latter fact at first sight appears scarcely adequate to explain the great delay in the divisions of B as compared with those of A, since between these two blastomeres the difference in size is comparatively slight. On the other hand, it must be remembered that since the macromeres have different *rates* of division the gap between the time of division of the four blastomeres may be considerably widened as the cleavage progresses; in other words, A may start with but a slight lead over B, yet it is, through its more rapid rate of division, enabled to increase that lead in subsequent divisions. This difference in the rates of division of the four quadrants will become obvious by reference to the table of cell-lineage at the back of the paper.

The spindles for the third cleavage are inclined strongly to the right, and the daughter cells, the first quartette of ectomeres, when first formed lie in the furrow between the macromeres. The diameter of these cells, as figs. 8 and 9 show, is about two-thirds of that of the macromeres from which they arose. They are not, however, all of the same size, the two posterior micromeres 1d and 1c being equal in size

and larger than the anterior pair 1a and 1b which also are equal. This size relation between the members of the first quartette occurs in *Nereis* (Wilson, 1892), *Amphitrite* and *Clymenella* (Mead, 1897), in *Arenicola* (Child, 1900), and, as the figures appear to indicate, in *Capitella* (Eisig, 1898), although Eisig states (p. 7) that the micromeres are "unter sich aber annäherend gleich grossen Zellen." Among the Mollusca this size relation seems not to appear at all.

The next cleavage, the fourth, is inaugurated by the second division of 1D. In fig. 8 this macromere is seen to be already in the prophase of division, and while the other cells in the ovum are preparing for division 1D separates by a leiotropic division into the relatively enormous cell 2d and the macromere 2D, which has now been reduced to the size of its fellows (figs. 9 and 10). 2d, the "first somatoblast" of von Wistinghausen (1891), which I shall label X, following Goette and most recent writers on the cell-lineage of annelids, lies in the second cleavage furrow slightly to the left of the mid-line. This cell is a most valuable aid to orientation, owing to its large size and fixed position. In this last division the macromere 2D, the smaller division product, becomes displaced, being crowded downwards so as to be almost directly below 1A (figs. 9 and 10). A similar displacement of 2D occurs also in *Unio* (Lillie, 1895), *Dreissensia* (Meisenheimer, 1901), *Capitella* (Eisig, 1898), and probably whenever the cell 2d greatly exceeds in size its parent macromere. 2D subsequently returns to the level of the other macromeres.

At about the time 2d is produced the first quartette undergoes a rotation in an anti-clockwise direction, so that each micromere comes to lie exactly over the macromere from which it arose (cf. figs. 8 and 10). This rotation is undoubtedly brought about by the division of 1D to form 2d, the latter cell being pushed backward and upward in a leiotropic direction. In *Crepidula* (Conklin, 1897), *Arenicola* (Child, 1900) and in other forms a similar rotation is brought about through the formation of the cells of the second quartette.

The micromeres 1d and 1c next divide, the spindles being inclined in a leiotropic direction. Through these divisions there are budded off from 1d and 1c, on their peripheral sides, small cells of equal size, $1d^2$ and $1c^2$, $1d^2$ being formed first (figs. 9 and 10). These cells, though formed by a truly spiral division, come to lie laterally to their parent cells and overhung by them (figs. 10, 11, and 12). A little later 1a and 1b follow in a similar division, the cells $1a^2$ and $1b^2$ being equal in size and somewhat smaller than $1d^2$ and $1c^2$, and come to lie in the furrow below the micromeres $1a^1$ and $1b^1$ and to the right of them. These

last-formed cells are minute and were at first difficult to detect, being overhung by their parent cells. The four cells $1d^2$, $1c^2$, $1a^2$ and $1b^2$ correspond both in origin and subsequent fate to the "trochoblasts" of annelids and to the "turret cells" of mollusks, and will be termed "trochoblasts," since the cleavage resembles that of the annelids rather than that of the mollusks.

While the trochoblasts are forming, 1A and 1C are in division (figs. 11 and 12), giving rise to two other members of the second quartette. The division of the two macromeres is nearly simultaneous, though C is slightly in advance. The two micromeres 2c and 2a are nearly equal in size to the two anterior cells of the first quartette.

Coincident with the division of 1A and 1C is the division of X (2d), the first step in the fourth cleavage (figs. 11, 12 and 13). X buds off a cell of about a third of its own diameter, low down on the right side (x^1, figs. 12 and 13). Closely following the formation of x^1 is the leiotropic division of 1B to form 2b, the last member of the second quartette, and the dexiotropic division of 2D to form the first member of the third quartette, 3d (figs. 13 and 16). Thus the macromere of the D quadrant has now obtained a lead of one division over the macromere of the B quadrant, and this lead is maintained as far as the formation of the fifth quartette. 3b is the smallest member of the second quartette, while 3d will prove to be the largest member of the third. Soon after 3d the other members of the third quartette appear one by one, 3c and 3a equal in size, and last 3b, the smallest of the four, which does not appear until after the formation of the mesoblast cell 4d (figs. 13 to 20).

Meanwhile divisions are occurring in the first and second quartettes. The divisions of the former are dexiotropic and concern the four larger cells only. The left products are nearly alike in size; smaller than the right products in the posterior quadrants, nearly equal to them in the anterior. There is thus formed a flat cap of eight cells (fig. 14), four of which lie radially and four interradially; the former, $1a^{1.2}$, $1b^{1.2}$, $1c^{1.2}$, $1d^{1.2}$, correspond in origin and very probably in fate to the "intermediate girdle cells" of *Nereis* (Wilson, 1892), and they will hereafter be called such. The four remaining cells, following the same terminology, will be called "stem cells." While these last divisions are in progress and the third quartette forming, 2a and 2c each buds off a small cell at its apex (figs. 15 and 16, $2a^1$ and $2c^1$). According to the rule of alternating cleavage this division should be leiotropic; in fact it is nearly equatorial, though the division of 2b, which occurs later, is strongly dexiotropic. The ovum represented in figs. 14, 15 and 16 is the only

one seen which shows this division in progress. However, the subsequent position of the cells $2a^1$ and $2c^1$ shows this division to be very nearly an equatorial one, though the next division of the cells $2a^2$ and $2c^2$ is strongly leiotropic (figs. 26 and 27). The cells $2a^1$ and $2c^1$ lie above their parent cells and in contact with the stem and intermediate girdle cells, at the same level with the trochoblasts. In consideration of their origin and subsequent fate they will be given the name "secondary trochoblasts," which is given by Mead (1897) to cells of similar origin and fate in *Amphitrite* and *Clymenella*. During these last divisions X has given rise to a cell on its left side x^2, in size similar to x^1, and nearly opposite that cell but at a somewhat higher level (figs. 16 and 18 and fig. 42). At the same time x^1 is also undergoing division, giving off a cell $x^{1.2}$ at its lower side. This is the so-called anal cell (Mead, 1897).

(4) *Segregation of Mesoblast.*

In the ovum represented in figs. 15, 16, and 17, a large spindle is seen in 3D. In fig. 18 the spindle is in the anaphase and the cell body has elongated, clearly indicating by the unequal size of its lobes the very unequal character of the division products. The latter are shown in fig. 24, the division having been completed. The posterior larger product is 4d, the chief mesoderm cell of mollusks and polychætous annelids. According to the law of alternating cleavage the spindle for this division should be leiotropic; in fact it is dexiotropic. This reversal in direction of the spindle can be accounted for partly by the crowding downward of 4d during the process of its formation. In the ovum represented in fig. 16 the spindle in 3B is nearly horizontal, while in that represented in fig. 18 it is inclined almost 40° to the horizontal plane. This inclination is clearly brought about by the great size of both 4d and X. Abutting as it does against X, 4d is prevented from attaining the level of 4D, and thus the left end of the spindle comes to be the lower. Why the spindle should not be inclined leiotropically at first is not clear.

The position of 4d in front of and below X and slightly to the left of the mid-line is almost precisely that of 4d in *Nereis* (Wilson, 1892), where the conditions concerned in its formation are very similar to those prevailing in *Dinophilus*.

4D is now reduced to about half the size of its fellows. This great reduction in size of the macromere 4D is not known among the annelids, but among mollusks it is a fairly common condition, *e.g.*, *Umbrella* (Heymons, 1893), *Unio* (Lillie, 1895), *Ischnochiton* (Heath, 1899), *Dreissensia* (Meisenheimer, 1901), *Trochus* (Robert, 1903).

With the formation of 4d and 3b the segregation of the germ layers now consists of 29 cells distributed as follows:

Ectoblast	First quartette,	12	cells.
	Second quartette,	8	"
	Third quartette,	4	"
Mesoblast	Fourth quartette (4d),	1	"
Entoblast	Entomeres,	4	"
	Total,	29	"

Thus in *Dinophilus* another example is already added to a long list of forms in which the ectoblast arises from the first three quartettes of micromeres, the mesoblast from the left posterior cell of the fourth quartette, and the entoblast from the remaining cells. To this mode of origin of the germ layers apparently the cephalopods alone form an exception. Among the annelids it is at present known to obtain

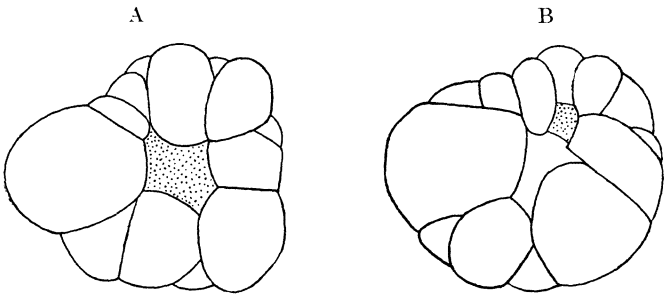


Fig. III, illustrating the reduction of the cleavage cavity. A, optical section of stage of about 54 cells; B, 72 cells. Cleavage cavity stippled.

only in the groups of the Polychæta and Echiuridæ. The fine paper of Eisig (1898) on *Capitella* seems to indicate that this manner of derivation of the germ layers does not extend to the Capitellidæ, since in *Capitella* Eisig derives the permanent mesoblast ("cœlomesoblast") from cells of the third quartette ($3c^1$ and $3d^1$), while 4d gives rise to larval or secondary mesoblast ("pædomesoblast") and ectoderm. However, in view of the probable tendency of the ova of *Capitella* to abnormal development, it is, I think, permissible to doubt these somewhat surprising results until they are verified in the same or some allied form. These results seem all the more surprising in view of the great resemblance which, in other respects, the cleavage bears to that of the Polychæta. Among the Echiuridæ, Torrey (1902, 1903) has found that *Thalassema* in its cleavage and in its mode of origin of the germ layers agrees closely with the Polychæta.

As compared with the Polychæta the origin of the mesoblast cell 4D in *Dinophilus* occurs very early. In fact the segregation of the three germ layers is completed at almost the same instant, since while 3D is dividing to form 4d, 2B is also dividing to form 3b. If, as Mead (1897) urges, the mesoblast belongs to the ideal 64-cell stage, then 4d should typically arise only after the 32-cell stage. As a matter of fact, it

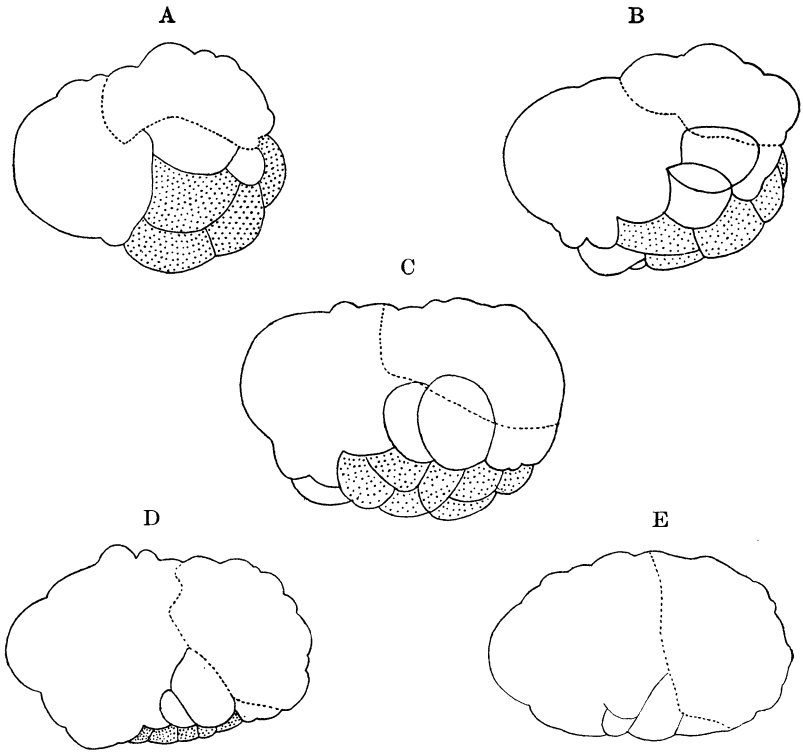


Fig. IV, A, B, C, D, E, diagrams of embryos of *Dinophilus* at different stages, illustrating the migration of the ectoderm during the closure of the blastopore. The ova are drawn as seen from the right side. The approximate limit of the first quartette is shown by a dotted line. The entoderm uncovered by ectoderm is stippled. From camera sketches.

does so arise in all the Polychæta whose early development has been investigated, and also in *Thalassema* (Torrey, 1902, 1903). On the other hand, in *Capitella* 4d belongs to the 29-cell stage, while among the Mollusca are several forms in which 4d is segregated before the 32-cell stage, *e.g.*, *Neritina* (Blochmann, 1882), *Crepidula* (Conklin, 1897), *Physa* (Wierzejski, 1897). At the other end of the series

stands *Ischnochiton* (Heath, 1899), in which the formation of 4d is delayed until the 72-cell stage. Yet, in spite of these modifications, which are plainly of a cœnogenetic nature, it is a remarkable fact, as Heath (1898) has pointed out, that the definitive mesoblast preserves its origin from 3D.

V.—Outline of the Further Development.

In order to make the histories of the quartettes more intelligible, it is desirable to give a brief account of the later history of *Dinophilus*, up to the time of hatching. My observations in regard to the main points agree essentially with the account given by Schimkewitsch (1895) for the White Sea form.

After the segregation of the germ layers, *i.e.*, at the 29-cell stage, a cleavage cavity of considerable size has been formed, and this cleavage cavity persists up to a stage of about 54 cells when it has reached its maximum size (text fig. III, A). The ovum is now roughly spherical in outline. Very soon, however, the ectoderm cells which roof the cleavage cavity spread out and flatten down, thus greatly reducing it, and finally causing it to be obliterated completely (text fig. IV, A and B). This flattening of the cap of ectoderm produces a pronounced change in the contour of the embryo, which is now decidedly elongated at right angles to the egg axis, *i.e.*, in an antero-posterior direction, and this elongation becomes more pronounced as development progresses. The ectodermal cap—that is, all of the ectoderm exclusive of the 2d group—now moves over the entoderm in a forward direction. The various stages of this movement are illustrated in text fig. IV, A, B, C and D. In the embryo A, the boundary of the first quartette, marked by a dotted line, lies in a plane nearly parallel to the long axis of the embryo; the apical rosette and the vegetal pole are still nearly opposite one another. In the embryo B the forward movement of the ectodermal cap can plainly be seen to have begun. Embryos C, D and E show the further movements leading to the closure of the blastopore, which brings the first quartette into a position at the anterior end of the embryo, after having rotated through an angle of about 80 degrees. That part of the ectoderm which formerly marked the animal pole, the apical rosette or its derivatives, is now just dorsal to the anterior pole of the embryo, while the boundary of the first quartette lies in a plane almost at right angles to the long axis of the embryo. This forward movement of the ectoderm is brought about by the mitotic activity of the cells of the 2d group dorsal to X—X, *viz.*, x^3, x^4–x^4, x^5–x^5, etc., and probably also of cells de-

rived from the intermediate girdle cell of the D quadrant. Mead (1897), Treadwell (1901) and Torrey (1903) have shown that in the annelids *Amphitrite, Podarke* and *Thalassema* respectively cells from this region wander out through the dorsal gap of the prototroch and contribute to the dorsal ectoderm of the trochophore. The thinness of the dorsal ectoderm of the *Dinophilus* embryo after the closure of the blastopore bears witness to the great mitotic activity in this region (compare figs. 54 and 56 and fig. 58). While this forward movement is taking place the ectoderm is increasing in lateral extent, as comparison of the embryos illustrated in text fig. IV readily shows. At the same time on the ventral side cells of the ventral plate have covered over the mesomeres, so that now the entoblast is completely enclosed with the exception of a small area at the vegetal pole, the blastopore. This soon closes, the ectodermal cells fusing so smoothly as to leave no visible trace of their union. Meanwhile the entodermal cells have undergone a change in that their lower or vegetal pole ends have become progressively smaller as the blastopore closes, while their upper or animal pole ends have become correspondingly larger. During this change in shape of the entomeres their nuclei recede from the surface and move inward (figs. 53–56).

The stomodæum appears as a shallow depression of the ectoderm at the point where the blastopore closed (fig. 58). The ventral plate now begins to grow forward very rapidly, pushing the stomodæum before it until the latter reaches a subterminal position, the position of the definitive mouth (fig. 59, st.). The stomodæal invagination now deepens from a shallow depression to a finger-like inpushing, directed somewhat backward. In fig. 59 the posterior wall of the stomodæum is seen to have thickened; this mass of cells (pro.) is the rudiment of the proboscis. In fig. 60 this organ (pro.) has assumed essentially its definitive structure and relations. From the stomodæal invagination is formed all of the alimentary canal anterior to the stomach, including the Vormagen or proventriculus.

While these changes are taking place the entodermal cells have been slowly dividing and have arranged themselves about a centrally situated cleft, the rudiment of the lumen of the future stomach and intestine (fig. 59, sto.l.). As the development progresses the entodermal cells assume the arrangement and appearance of an epithelium, while at the same time the lumen of the future stomach increases in extent. In fig. 60 the entoderm cells are now seen to form a cuboidal epithelium, while the lumen of the stomach is a long and narrow cleft, extending obliquely downward and forward. From the posterior end of the

stomach projects a short diverticulum, the rudiment of the intestine (int. l.).

The proctodæum appears, at the time that the stomodæum has assumed its final position, as a simple thickening of the ectoderm at the posterior end of the body (fig. 59, pr.). At a later stage a small depression appears in this thickening (fig. 60, pr.). The proctodæum probably forms only the terminal portion of the intestine, or anus. Fusion of the proctodæum and stomodæum with the entodermal portion of the alimentary tract does not take place until very shortly before hatching.

The rudiment of the brain appears early. Just after the blastopore has closed, the ectoderm at the anterior end of the embryo is thicker than elsewhere, presenting the appearance shown in optical section in fig. 58. The cells of this region multiply rapidly, and the ectoderm here soon becomes many-layered (fig. 59). At a later period (figs. 60 and 61) a transverse band of nerve fibres (br.com.) is seen below and in contact with the ectoderm at this point, while the nuclei of the deeper layers have become very numerous. The latter undoubtedly constitute the nuclei of the ganglion cells, while the band of nerve fibres constitutes the commissure connecting the lateral lobes of the brain of the adult. The brain is now essentially similar to that of the adult, which remains throughout life in contact with the ectoderm of the head.

The further changes before hatching consist principally in the elongation of the body and the formation of somites. The rapid growth of the ventral plate causes the embryo to bend strongly toward the dorsal side, as is shown in fig. 60, though here the flexure is not nearly so great as it becomes at a still later period. This rapid growth of the ventral ectoderm is apparently not compensated for by a corresponding growth of the dorsal ectoderm until shortly before hatching. The head is separated from the trunk by a constriction at a stage corresponding to that illustrated in fig. 59, that is, as soon as the stomodæum has taken up its final position at the anterior end of the embryo. The trunk segments do not appear until the body begins to elongate, when the constrictions which mark them off appear successively from in front backward. In the embryo shown in horizontal section in fig. 61, one trunk segment, the first, is clearly shown, while two more are indicated posterior to this one.

VI.—History of the First Quartette.

(1) *The Cross and the Intermediate Girdle Cells.*

At the time of the formation of the mesoblast cell 4d, the first quartette consists of twelve cells—four stem cells, four intermediate girdle cells and four trochoblasts (fig. 14). According to the ideal scheme the trochoblasts should by this time have divided once, but their rate of division is very greatly retarded, obviously in correlation with the comparatively late period at which they become functional.

The stem cells are the next to divide. Even before 4d is formed, the posterior stem cells ($1d^{1.1}$ and $1c^{1.1}$) are preparing for a leiotropic cleavage (fig. 14). These divide and $1a^{1.1}$ and $1b^{1.1}$ soon follow; the upper (left) products resulting from the division are four small cells of equal size, forming a quatrefoil at the animal pole, the "rosette" of *Nereis* (Wilson, 1892) and other annelids (fig. 20).

While the rosette is forming, the intermediate girdle cells are also engaged in a leiotropic division which is unequal in all save the D quadrant (fig. 20). In the A, B and C quadrants the right and peripheral product is a small cell, to which, in consideration of its origin and probable fate, has been given the name "accessory trochoblast," a term applied by Heath (1899) to cells of *Ischnochiton* of similar origin and fate.

While the division of the stem cells and intermediate girdle cells is in progress the trochoblasts also divide (figs. 20, 21, 22 and 29). $1c^2$ and $1d^2$ usually divide at nearly the same time, although in fig. 14 $1c^2$ is the only trochoblast showing a spindle; $1a^2$ follows more or less closely on $1d^2$ and $1c^2$, while $1b^2$ is always delayed. The division is meridional in the posterior trochoblasts; in the anterior pair the plane of division, although very nearly meridional, is nevertheless inclined sufficiently to indicate a dexiotropic cleavage (fig. 29).

Returning to the stem cells, the posterior pair of these are found to be again in mitotic activity, their spindles having reached the metaphase before the last division of $1b^{1.1}$ is fully completed (fig. 20). These spindles are not spiral, but truly radial in position. Their central ends lie at a higher level than their peripheral ends.

This division marks the beginning of bilateral cleavages in the first quartette, though these do not appear in the intermediate girdle cells. In fig. 20 the symmetrical position of the spindles in $1c^{1.1}$ and $1d^{1.1}$ is obscured owing to the clockwise rotation of the first quartette, caused by the formation of x^3 in a dexiotropic direction. In fig. 25 may be seen the products of this division: of these the peripheral products are slightly the larger, and are overlapped by the central products,

owing to the oblique position of the spindle. Meanwhile the first quartette has rotated in an anti-clockwise direction, being thus restored to its original position. Next $1a^{1.1.2}$ and $1b^{1.1.2}$ divide, the spindles in their cells being also truly radial, with the central ends the higher. The cell pattern produced by these divisions is similar to that first found by Wilson (1892) in *Nereis*, *Polymnia*, *Spio* and *Aricia*, and called by him "the cross." The characteristic feature of the cross is the radial divisions of the stem cells. This, as has just been described, is also the case in *Dinophilus*, but the cell pattern is marred by the belated division of $1b^{1.1.2}$, which does not occur until the basal cells of the posterior arms of the cross are again in division (fig. 30). The cross is formed also in *Amphitrite*, *Clymenella* and *Lepidonotus* (Mead,

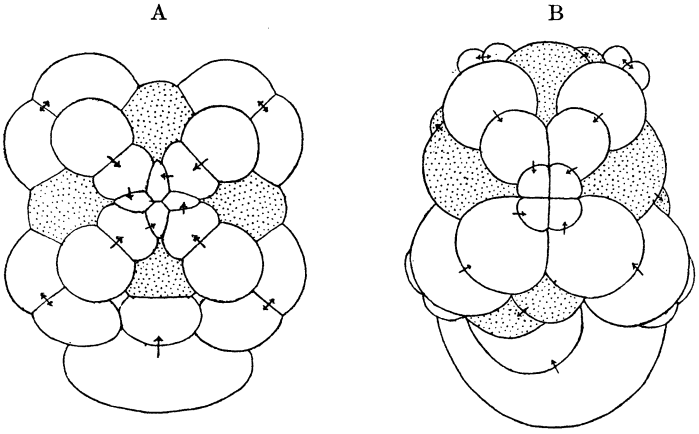


Fig. V, A, the "cross" of *Nereis*, copied from Wilson (1892); B, diagram of the "cross" of *Dinophilus*. The four quadrants are represented as having divided simultaneously.

1897), *Capitella* (Eisig, 1898), *Arenicola* and *Sternaspis* (Child, 1900), and *Podarke* (Treadwell, 1901). In *Podarke* the spindles are slightly dexiotropic, and in *Chætopterus* (Mead, 1897) they are so much so that the cross is not formed at all. In *Dinophilus* the spindles are truly radial, no trace of the spiral type having been observed. The comparison of the *Dinophilus* cross and that of the polychætous annelid *Nereis* will be made clear by reference to text fig. V, A. and B.

The radial spindles forming the cross mark the appearance of bilateral cleavage in the first quartette. Not only is the cross in itself a symmetrical structure, but it is bilaterally symmetrical with respect to the median plane of the embryo, and this is, as Wilson pointed out in *Nereis* (1892), "an adult bilaterality foreshadowed, long before

bilateral divisions begin, in the arrangement of the cells." In other words, the adult bilaterality is expressed in the arrangement of the four cells $1a^{1.1.2}$, $1b^{1.1.2}$, $1c^{1.1.2}$ and $1d^{1.1.2}$ brought about through spiral cleavages, and in fact foreshadowed in the 8-cell stage. These facts have all been shown to be true for all the forms in which a cross occurs, and need not be dwelt upon here.

While the anterior stem cells are dividing to form the anterior arms of the cross, new spindles appear in the intermediate girdle cells $1a^{1.2.1}$ and $1c^{1.2.1}$ (figs. 25, 26 and 27). These cells then give rise at their peripheral margins to tiny cells with a deeply staining nucleus (figs. 30, 31 and 32). The direction of these divisions is somewhat uncertain, but judging by the position of the smaller products it is dexiotropic in $1a^{1.2.1}$ and leiotropic in $1c^{1.2.1}$. Later $1b^{1.2.1}$ gives rise to a similar cell (figs. 37 and 41). This division is unmistakably dexiotropic. It is of interest to note that in *Amphitrite* (Mead, 1897) and *Arenicola* (Child, 1900) these same cells should also be small and provided with a densely staining nucleus. In *Thalassema* (Torrey, 1902, 1903) these cells are truly rudimentary, and it seems quite probable that such is the case in *Dinophilus* also, judging from their minuteness and staining reactions.

Closely following the division of $1b^{1.1.2}$ to form the right anterior cross arm, indeed almost simultaneous with this division, is that of the posterior basal cells of the cross. The plane of this division is meridional, as is also the case in the annelid cross, but differs from the latter in that the division is an asymmetrical one, since the left product of the left posterior basal cell is very much smaller than the right product, while the products of the right posterior basal cell are equal (figs. 30 and 37). This curious divergence from the annelid type I am at present unable to explain. Possibly a further study of the cleavage and of the history of the individual cells of the cross might offer a solution of the problem. Closely following the divisions of the posterior basal cells comes the division of the posterior terminal cells. The spindles for this division are meridional, and the upper and central products of this division are minute cells, which lie above their parent cells as shown in figs. 37, 38 and 39. These correspond in origin to the "nephroblasts," which in *Nereis* (Wilson, 1892) form the head kidney (or possibly slime glands), and which in *Amphitrite* (Mead, 1897) give rise to the large mucous glands of the umbrella of the trochophore. In *Podarke* (Treadwell, 1901) these cells are also minute and occupy relatively the same position as in *Dinophilus*. Treadwell describes small cells of the first quartette which sink through the ectoblast and,

reaching the cleavage cavity, there degenerate. Torrey (1902, 1903) suggests that among these small cells are those two mentioned above, viz., $1d^{1.1.2.2.1}$ and $1c^{1.1.2.2.1}$. In *Clymenella* the nephroblasts are also small and form part of the dorsal ectoderm of the head, as they do in *Arenicola*. What the fate of these cells is in the case of *Dinophilus* I do not know.

The significance of the annelid and molluscan crosses and of bilateral cleavage in general has been most thoroughly discussed by recent writers on cell-lineage, and on this particular point I have nothing to add. Since, however, it is generally agreed that bilateral cleavages are not directly referable to purely mechanical causes, but are the result of unknown factors, which cause the throwing back of the bilaterality of the adult upon the embryo, it is remarkable that this appearance of the adult bilaterality should have occurred in the same cell generation, in the same direction, and resulting in the same cell pattern as in the Polychæta. This resemblance is still further continued in the direction of division of the posterior cross arms.

The rosette cells at the time of their formation lie in the furrows between the stem cells, but almost immediately afterward they move in a clockwise direction (cf. $1a^{1.1.2}$ and $1a^{1.1.1}$ in fig. 20), so that the cells now lie interradially, instead of radially, as in the annelid cross (text fig. V, B).

At a stage numbering between one hundred and one hundred and fifty cells, the rosette divides dexiotropically and equally. Its cells, which possibly later undergo another division, form a cluster of cells much smaller than those surrounding it, and can be thus distinguished nearly to the time of the closure of the blastopore. As far as my observations on both fixed and living material extend, the rosette never bears cilia. In *Capitella* also an apical tuft of cilia is never present, though the rosette is formed as in other Annelida.

The further history of the first quartette is comparatively simple. The forward movement of the cells of the first quartette has already been described (see text fig. IV, A–E). The limits of the first quartette have been determined by two landmarks, the cells $2a^{2.2.1}$ and $2c^{2.2.1}$ on the right and left sides respectively, and that chain of small cells which I have identified as the prototroch. These latter extend over the sides, passing just anterior to the cells $2a^{2.2.1}$ and $2c^{2.2.1}$, and around the ventral side as a continuous row, but on the dorsal side there is the wide gap so often found in the early stages of the trochophore larva, and due to the same cause, viz., the non-participation of the products of 2d in the formation of the prototroch. Owing to this

fact I have not been able to trace with certainty the dorsal posterior limits of the first quartette. On the ventral side the boundary of the first quartette is indicated in fig. 55 by the most anterior row of small cells, and curves forward in a semicircle in front of the blastopore. The position and general outline of the first quartette are sufficiently indicated in text fig. IV, E. It covers like a cap the anterior end of the embryo and corresponds to the umbrella of the trochophore.

At the time of the appearance of the stomodæum the ectoderm at the anterior end of the embryo is seen (fig. 58) to consist of high columnar cells, while the ectoderm cells covering the rest of the embryo—except at the posterior end, where are the still large remnants of the X cells—are cubical or flattened. The centre of this thickened area is not precisely the anterior pole of the larva, but slightly dorsal to that point, at which place the descendants of the rosette were last recognized. This thickening is the rudiment of the brain of the adult. At a later stage the cells of the thickened area have multiplied and become so closely crowded together that their outlines are barely distinguishable. In fig. 59 the brain rudiment has increased both in thickness and extent, and has at the same time moved somewhat dorsad owing to the rapid growth of the ventral plate. In fig. 58 the thickened area represents, as comparison with text fig. IV, E, will show, less than half of the first quartette; in fig. 59 it has increased to nearly twice its former area. Fig. 60 shows the brain in sagittal section at a slightly later stage. On the dorsal side, just behind the brain, is a deep furrow which in the adult separates the head from the trunk. The lateral extent of the brain is shown in fig. 61, a horizontal section of a stage more advanced than that shown in fig. 60. In this embryo the brain already shows signs of its bilobed character. From these figures it can be seen that nearly the whole of the first quartette is involved in the formation of the brain, if we assume that the second preoral ciliated band of the adult arises from the trochoblasts, as is probably the case, since the brain rudiment is limited posteriorly by the constriction which separates the head and trunk; while the second preoral ciliated band of the adult (prototroch) appears on the elevation of the head just anterior to this constriction.

The cell origin of the cerebral ganglia among the mollusks has been very fully described by several recent investigators (Conklin, 1897; Holmes, 1900; Meisenheimer, 1901; Robert, 1903); among the annelids by von Wistinghausen (1891) and by Wilson (1892) for *Nereis*, by Mead (1897) for *Amphitrite*, by Eisig (1898) for *Capitella*. Wilson has shown that von Wistinghausen's account was incomplete and

erroneous, and believed that the cerebral ganglia arise from the cross. Later investigators have been unable to either prove or disprove this statement. It is at least certain that the brain is formed from the first quartette of micromeres, and its first rudiment is found in the neighborhood of the rosette. These facts, however, can be gathered from the works of the older investigators (Hatschek, Salensky, *et al*).

In *Dinophilus* the rudiment of the cerebral ganglia involves more than the cells of the cross alone. Its development, however, is typically annelidan, since the ectodermal thickening which represents the earliest rudiment of the brain appears beneath the rosette, which in the trochophore bears the apical tuft of cilia. *This rudiment then is to be regarded as the ontogenetic representative of the "Scheitelplatte" of the annelid trochophore.*

(2) *The Prototroch.*

The head of both the larval and adult individuals of *Dinophilus gyrociliatus* (*apatris*) is encircled by two narrow transverse bands of long cilia, similar to those which are found on the metameres of the trunk, as illustrated in text fig. VI. Both of these bands are preoral; the first being situated just anterior to the eyes, the second surrounds the head near its juncture with the trunk, and passes ventrally just anterior to the mouth. This second preoral band corresponds in position and function to the prototroch of the trochophore. It first appears at a stage nearly corresponding to that figured in fig. 59 as two delicate tufts of cilia on each side of the head.

The history of the cells composing this second preoral ciliated band, which I have already taken the liberty of calling the prototroch, will be considered under the head of the first quartette. I have done this chiefly because of the important place given in the literature of cell-lineage to those components of the annelid prototroch and the molluscan velum derived from the first quartette, the "primary trochoblasts" (Mead, 1897).

At the 29-cell stage the primary trochoblasts are already beginning to divide (figs. 14 and 15). The division is equal in all. The posterior pair divide meridionally, while in the anterior pair the spindle is inclined from the horizontal plane in a dexiotropic direction. While the posterior pair begin to divide as early as the stage shown in fig. 14 (26 cells), $1b^2$ does not complete its division until a stage numbering 54 or more cells is reached. Meanwhile the cells of the second quartette in the A and C quadrants have each given off a small cell above, by a nearly equatorial cleavage (figs. 14, 15, 16, 18 and 19). 2b later also divides, but this cleavage, instead of being equatorial, is strongly dexio-

tropic, as it should be, following the law of alternating cleavage (fig. 29). While this division is in progress, the lateral cells of the second quartette are again in division (figs. 26 and 27). This division is leiotropic; its result is that a cell similar to but smaller than $2a^1$ and $2c^1$ is placed to the left of these cells. Lastly, $2b^2$ divides similarly, the products being shown in fig. 41. For the six cells thus formed from the second quartette, $2a^1$, $2a^{2.1}$, etc., I have adopted Mead's name of "secondary trochoblasts," though they are not precisely identical in origin with those to which Mead gave this name. At a stage of about 81 cells the secondary trochoblasts $2a^1$ and $2c^1$ divide leiotropically and equally (figs. 34 and 35). No doubt $2b^1$ also follows suit, though I have not seen this division.

The origin of the "accessory trochoblasts" $1a^{1.2.2}$, etc., by the dexiotropic division of the intermediate girdle cells has already been described. There is now an irregular row, formed of 22 small cells, which encircles the embryo at a level just between the first and second quartettes. These cells are arranged as follows, passing from left to right: $1d^{2.2}$–$1d^{2.1}$–$2a^{2.1}$–$2a^{1.2}$–$2a^{1.1}$–$1a^{2.2}$, etc. It is very probable that the accessory and primary trochoblasts soon divide again, since in the embryo from which fig. 50 was drawn $1c^{2.2}$ and $1c^{1.2.2}$ were both undergoing an equal and meridional division. The row of cells thus formed is clearly distinguishable up to the closure of the blastopore, and it was by means of this row as well as by the large size of $2a^{2.2.1}$ and $2c^{2.2.1}$ that I have been able to trace the boundary of the first quartette, as shown in text fig. IV, A. The trochoblasts are very transparent, and become elongated in the direction of the cell row.

At the stage shown in fig. 55 the prototroch is nearly transverse to the long axis of the embryo, as is also shown by the dotted line in text fig. IV, E, but in front of the blastopore it bends sharply forward in a semicircle. In these figures the trochoblasts are represented by the most anterior row of that group of small cells lying just anterior to the blastopore. Beyond the stage represented in fig. 55 I have not been able to trace this group, since all the cells of the ectoderm, through rapid mitosis, soon become nearly uniformly small in size. In fig. 44, where the trochoblasts were last identified, they were on the ventral side, but a short distance anterior to the blastopore, and passed up on each side in a row nearly transverse to the long axis of the embryo (cf. text fig. IV, E). Since the stomodæum appears at the point where the blastopore closed and is then pushed forward through growth of the ventral ectoderm (ventral plate), it follows that the trochoblasts must still remain as a more or less transverse row passing in front of the stomodæum. At the close of the embryonic life, when the trans-

verse ciliated bands characteristic of the embryo are acquired, among these is one which passes around the head just in front of the mouth, and occupying precisely the position that the row of trochoblasts would be expected to assume after the growth changes which have taken place. It must either be supposed that the ciliated ring in question is derived from the trochoblasts or else from other cells occupying a very similar position. Of the two hypotheses the latter appears to me much the more probable, especially in view of the persistence of the prototroch in many annelid larvæ, where it encircles the head, passing ventrally just anterior to the mouth, its position thus essentially coinciding with that of the second preoral ciliated band of *Dinophilus*.

The cell origin of the annelid prototroch has been determined in *Nereis* (Wilson, 1892), *Amphitrite* and *Clymenella* (Mead, 1897), *Arenicola* (Child, 1900) and *Podarke* (Treadwell, 1901). Among the mollusks the precise cell origin of the velum has been determined in but two forms, *Ischnochiton* (Heath, 1899) and *Trochus* (Robert, 1903), although in two other forms, *Crepidula* (Conklin, 1897) and *Planorbis* (Holmes, 1900), the origin of the velum has been determined with considerable, if not absolute, certainty. Below is given, for the sake of convenience in comparison, a table of the components of the prototroch and velum in the A quadrant of those forms in which it has been most carefully worked out. The components of the prototroch of *Dinophilus* are added for comparison.

	Primary Trochoblasts.	Secondary Trochoblasts.	Accessory Trochoblasts.
Nereis	$1a^{2}$	None	None
Amphitrite, *Clymenella*, *Lepidonotus*, *Arenicola*	$1a^{2}$	$2a^{1.1.1}$, $2a^{1.1.2}$, $2a^{1.2.1}$	None
Podarke	$1a^{2}$	$2a^{1.1.2}$, $2a^{1.2.1}$	$1a^{1.2.2.2}$
Thalassema	$1a^{2}$	$2a^{1.1.1}$, $2a^{1.1.2}$, $2a^{1.2.1}$	$1a^{1.2.2.2}$
Ischnochiton	$1a^{2}$	$2a^{1.1.1}$, $2a^{1.1.2}$	$1a^{1.2.2.2}$
Trochus	$1a^{2}$	$2a^{1.1.1}$, $2a^{1.1.2}$, $2a^{1.2.1.1.1}$	None
Dinophilus	$1a^{2}$	$2a^{1.1+}$, $2a^{1.2+}$, $2a^{2.1+}$	$1a^{1.2.2+}$

All the forms mentioned have the primary trochoblasts in common; in all, except *Nereis*, the gaps between the four groups of primary trochoblasts are closed partly or entirely by cells derived from $2a^1$, $2c^1$ and $2b^1$; *Ischnochiton*, *Podarke* and *Dinophilus* agree in that the cells $1a^{1.2.2}$, $1b^{1.2.2}$ and $1c^{1.2.2}$ participate in the prototroch. In common with the annelids, there is also a dorsal gap in the prototroch, owing to the fact that none of the cells of the D quadrant, except the primary trochoblasts, take part in its formation.

Dinophilus differs from all the other forms in (1) the small size of the primary trochoblasts, (2) that at least the posterior pair of the primary trochoblasts probably divide twice meridionally, and (3) in that $2a^{2.1}$, $2b^{2.1}$ and $2c^{2.1}$ also take part in closing the gaps between the groups of primary trochoblasts in the quadrants A, B and C. These differences, however, are of slight importance compared with the great and striking similarity to the annelids manifested in the origin of the cells which almost certainly form the second preoral ciliated band of the adult *Dinophilus*. In the light of this similarity the conclusion is almost unavoidable *that the second preoral ciliated band of Dinophilus is truly the homologue of the annelid prototroch.*

The peculiarities in the formation of the *Dinophilus* prototroch become readily comprehensible if the character of the end result, *i.e.*, the second ciliated band, be considered, and also the time at which this organ comes into functional activity. The cleavages involved in the formation of the prototroch are thus clearly seen to be of prospective significance, or morphogenetic.

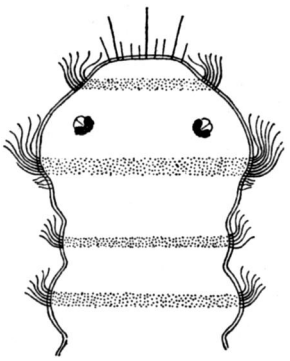


Fig. VI, head and first two trunk somites of *Dinophilus* larva, soon after hatching. Drawn from a living individual

The ciliated bands of the larval or adult *Dinophilus* are, as compared with the prototroch or velum of such forms as *Amphitrite*, *Arenicola*, *Podarke*, *Ischnochiton* or *Trochus*, relatively narrow tracts (text fig. VI), consisting of but a few rows of long cilia, as shown in the figures of Korschelt (1882) or Meyer (1887). Moreover, these tracts probably do not become functional until late in embryonic life. Conklin (1897) has shown that the size of the protoblast of an organ is related not only to the size of that organ, but also to the time at which it becomes functional. In the light of this fact the small size of all the trochoblasts is easily explained.

The two meridional cleavages of the primary trochoblasts and the participation of $2a^{2.1}$, $2b^{2.1}$ and $2c^{2.1}$ in the prototroch tend toward the same end, *i.e.*, the production of a narrow band of small cells. 2a, 2b and 2c, instead of each giving off one large cell to the prototroch which subdivides into four cells, covering a broad area, each lays down side by side two small cells, which may then divide meridionally (or nearly so) without violation of the law of alternating cleavages, and thus increase the length of the prototroch, but not its breadth.

VII.—History of the Second and Third Quartettes.

(1) 2*d* (=*X*).

The origin of 2d (=X) has already been described. The size of this cell is enormous as compared with that of the other cells of the embryo. This relatively enormous size recalls the conditions found among the lamellibranchs, *Cyclas* (Stauffacher, 1893), *Unio* (Lillie, 1895) and *Dreissensia* (Meisenheimer, 1901), and among annelids in *Arenicola* (Child, 1900), and also in *Clepsine* (Whitman, 1878). The first two divisions of this cell have already been described; the products of these divisions and the first product of the third division, x^3, are shown in fig. 42. In this figure it is seen that x^1 and x^2 are not precisely symmetrical with respect to X, either in size or position, since x^2 is somewhat larger than x^3 and placed at a higher level on X. $x^{1.2}$ lies precisely in the ventral mid-line. x^3 lies dorsally to the left, as viewed from behind, and has been formed by a dexiotropic division of X. The approaching division of $x^{1.1}$ and $x^{1.2}$ as shown in the figure is of interest, since $x^{1.1}$ is the first cell in the embryo to violate the law of alternating cleavages, inasmuch as the spindle for this division has the same direction as that which formed $x^{1.2}$. This reversal of the spindle in $x^{1.1}$ was first pointed out by Mead (1897) for *Amphitrite*, and was also found by Child (1900) in *Arenicola*. The products of this division and the corresponding one of x^2 are shown in fig. 43. In this figure are also shown the spindles for the fourth cleavage of the X group. Two points are to be noted in this cleavage: (1) the division of x^2 has evidently pushed x^3 toward the left (right in the figure), bringing it almost into the mid-line; and (2) X is dividing into bilaterally placed halves. This bilateral division of X is a striking and constant feature of the unequal type of cleavage among the polychætous annelids. A similar division occurs in the lamellibranchs, but appears at the fifth cleavage, instead of at the fourth. All the products of the fourth cleavage of X are shown in fig. 44. $x^{1.1.2}$ has budded off a small cell below; $x^{2.2}$ has divided into nearly equal parts by a meridional cleavage; while x^3 has split into

unequal parts, the left product being the greater, and occupying a median position in the cleft between X and X. The cell $x^{2.2.1}$ is now nearly equal to $x^{1.1.2.1}$, and their next two divisions (figs. 47 and 49) are bilaterally symmetrical. The same may be said of the small cells $x^{1.1.1}$ and $x^{2.1}$ (figs. 41 to 48). While I have not followed the history of these latter cells further, it is extremely probable that their divisions are also bilaterally symmetrical. There is here then an instance of a symmetrical arrangement of cells derived by asymmetrical divisions, examples of which are found in the annelid and molluscan crosses, and in the X group of *Amphitrite* (Mead, 1897) and *Arenicola* (Child, 1900), and probably also in many other forms. While $x^{1.1.2.1}$ and $x^{2.2.1}$ are almost equal, $x^{2.2.1}$ is really somewhat the smaller of the two; and in this connection it is of interest to remember that on this side, the left, next to $x^{2.2.1}$, lies the largest member of the third quartette, viz., 3d. Returning to figs. 44 and 45, the cells $x^{1.1.1}$ and $x^{2.1}$ are seen to have each given off a minute cell toward the vegetal pole. These cells are also shown in figs. 38 and 39.

The products of the bilateral division of X, called by Wilson (1892) the "posterior proteleblasts," and by Child (1900) the "posterior stem cells," undergo a series of bilateral divisions. The first is shown in figs. 45–47, x^4 and x^4. These cells have pushed forward $x^{1.1.1.1}$ and $x^{2.1.1}$. The cells formed by the next division of the posterior stem cells come to lie together near the median plane, and are also budded off toward the animal pole. Meanwhile $x^{3.2}$ has been divided into equal parts by a meridional plane, and these three cells assume a symmetrical arrangement (fig. 48). I regret that I have not followed the division of the members of the X group beyond this point. Figs. 53 and 55 show what is probably the next division of the posterior stem cells, by means of which two large cells, x^6–x^6, are separated off ventrally and laterally. After the closure of the blastopore the posterior stem cells each undergo a further subdivision into two cells by a meridional cleavage. The four cells thus formed resemble in appearance the "posterior teloblasts" of *Nereis* (Wilson, 1892).

The lineage of those cells which lie on the vegetal pole side of the posterior stem cells was followed as far as is indicated in the figures, but a consideration of their cleavages is of little value, inasmuch as there is no sign of a paratroch at the time when the blastopore closes, and the cells on the ventral side of the embryo have by this time become so small and thin as to be very difficult to distinguish satisfactorily.

There are two points in connection with the cleavage of X which require special mention. The first of these concerns the first four

cleavages of X. These cleavages are essentially the same as those of X in *Amphitrite* and *Clymenella* (Mead, 1897) and *Arenicola* (Child, 1900). This similarity extends not only to the direction of the cleavages, but also to their products, which bear to one another a very similar size relation. This striking resemblance cannot be ascribed to the effect of alternating cleavages, since at the third cleavage this law is violated in $x^{1.1}$, at the fourth in X. The divisions of X in *Dinophilus* and *Nereis* (Wilson, 1892) differ no more than do the corresponding divisions in *Nereis* and other annelids (*Amphitrite*, etc.). These differences are, that in *Nereis* x^3 is formed exactly in the dorsal mid-line, and that the division of x^1 is delayed, and is nearly equal when it occurs. In all the Polychæta whose cytogeny is known (except *Podarke*) bilaterality appears in X at the fourth cleavage. It may then be said of *Dinophilus that bilaterality appears in the cell X in the same cell generation as in all polychætous annelids investigated having the unequal type of cleavage, and appears in* x^1 *at the same cleavage as in at least three polychætous annelids.*

The second point concerns the arrangement of the cells of the X group. In *Nereis* the main body of the descendants of X are so distributed that they come to lie on the vegetal pole side of the stem cells, the latter remaining near the prototroch. In the other Polychæta, up to the time of the closure of the blastopore, the descendants of X are uniformly distributed about the posterior stem cells, so that up to a late stage the latter occupy a central position with regard to their products. In *Dinophilus*, on the other hand, the greater part of the descendants of X, up to the time when the blastopore is closed, are distributed dorsal and lateral to the posterior stem cells. This peculiarity is related to the peculiar shifting of areas, which has already been briefly described in the chapter on the later development. This distribution is not due entirely to a difference in the direction of the division of X—though in *Nereis* two cells, x^4 and x^4, are budded off toward the vegetal pole—but to a shifting of the cells among themselves. For example, in *Amphitrite* the cells x^1 and x^2 shift to the vegetal pole side of X, while in *Dinophilus* the same cells always retain a lateral position.

The further history of this group, as far as I have been able to trace it, is as follows. At a time near the closure of the blastopore, as shown in fig. 55 and text fig. IV, E, the first quartette has been shifted forward through nearly 90 degrees, being pushed forward through the formation of x^4–x^4, x^5–x^5, etc., until the descendants of these cells cover the dorsal surface of the embryo up to the limits of the first quartette and

also cover a large portion of the lateral surface. Behind the blastopore the descendants of X form a small group of cells, which in fig. 53 have not yet covered in the primary mesoblasts. Mitotic activity in this region has been very slight, as may be gathered from examination of the optical section shown in fig. 54. The effect of active mitosis in the posterior dorsal region is perceived in the thinness of the ectoderm in this region. The X cells posterior to the blastopore appear to advance and fuse with the other ectoderm cells surrounding the blastopore. Whether they actually form part of the blastopore I cannot at present say, but judging from their position in fig. 55 it seems probable that they do. The stomodæum is formed, as has already been stated, at precisely the point where the blastopore closed. It then moves rapidly forward to occupy the position shown in fig. 59. This movement is caused by the growth of the cells posterior to the blastopore, which up to this time have been slow in dividing, as well as by new additions from the posterior stem cells. This ventral plate of cells derived from X corresponds in origin and position to that group known among the annelids as the "ventral plate." Examination of the ventral side of an embryo at this stage shows numerous mitotic figures, and the rapid growth of this region is testified to by the thinning of the ventral ectoderm and by the dorsal flexure shown in figs. 59 and 60.

From this time on, it is evident that the further growth of trunk ectoderm is due entirely to growth of the cells of the X group, for, since the appearance of the segments from in front backward indicates terminal growth, it is evident that the descendants of X can alone be concerned. 2d or X, then, contributes at least the larger part of the trunk ectoderm, as is the case in all the polychætous annelids whose cell lineage has been studied. A larva of four segments is shown in horizontal section in fig. 61. By comparison of this figure, which is drawn to the same scale as the other figures, an adequate idea can be obtained of the relatively enormous extent of the trunk ectoderm. When it is recollected that this latter is formed principally, if not exclusively, from 2d, without any addition of food material from the exterior, the colossal size of 2d is very readily comprehended. A very similar case is found in the development of *Arenicola*. In this form, as already remarked, the first somatoblast is also unusually large, and furnishes material for the first three segments of the adult worm. In *Dinophilus* direct development has wholly supplanted the larval type, since the material stored up in 2d forms six body segments, the total number found in the adult.

(2) *2a, 2b and 2c and the Third Quartette.*

The divisions of the cells 2a, 2b and 2c up to an advanced stage have been described in dealing with the history of the prototroch. There is left but one division to record, which I have observed only in $2a^{2.2}$ and $2c^{2.2}$. This division is shown in figs. 34 and 35. The plane of the division is nearly equatorial, but inclined slightly in a leiotropic direction in the C quadrant, in a dexiotropic direction in the A quadrant. Of the two products the lower is somewhat the smaller. I have never witnessed a corresponding division in $2b^{2.2}$, but it very probably occurs. The position and small size of this cell make it difficult to study in the later stages. The two products of $2a^{2.2}$ and $2c^{2.2}$ respectively are quite conspicuous up to an advanced stage, although they probably divide into smaller cells just before the blastopore closes.

The origin of the third quartette has been dealt with in the description of the cleavages. 3d is the largest cell in this quartette, 3b the smallest, while 3a and 3c are intermediate in size. At the 40-cell stage 3d divides equally and leiotropically. The products of this division are shown in fig. 22. A little later 3c divides, but in quite a different manner, budding off a small cell toward the animal pole. The plane of the division is equatorial (fig. 26). Next 3a also buds off a cell toward the animal pole, the spindle being nearly vertical, though the position of the products indicates a leiotropic division. 3b divides some time after 3a, and here the spindle is decidedly leiotropic (fig. 34). It is interesting to note that both 3b and 2b, in their respective quartettes, are the most conservative in retaining the primitive direction of their divisions. While 3a is in division, spindles are seen in $3d^1$ and $3d^2$ (fig. 27). These cells bud off tiny cells toward the animal pole (fig. 32). Of these latter that derived from $3d^1$ is the smaller. The spindles for this division are nearly vertical, but sufficiently inclined to make the division leiotropic, in direct violation to the law of alternating cleavages. Even before 3b has completed its first division 3c has budded off another cell toward the animal pole, the spindle being inclined dexiotropically, as it should be according to the law of alternating cleavages (fig. 34). With this division, or rather the one which follows it, *i.e.*, that of 3b, I have ceased to follow the cell-lineage of the entire egg.

In fig. 53 the position of the cells $2a^{2.2.1}$, $2a^{2.2.2}$, $2c^{2.2.1}$, and $2c^{2.2.2}$ is clearly seen. Each pair of cells form a portion of the lateral margin of the blastopore; $2a^{2.2.1}$ and $2c^{2.2.1}$, which formerly were above, that is, to the animal pole side of $2a^{2.2.2}$ and $2c^{2.2.2}$, now lie anterior to them. They still, however, maintain their relative position in the ectoderm,

and their changed relation to the entoderm and the X group is the result of the shifting of the ectoderm already described. A clear idea of the change of position of the cells in question may be obtained from a glance at text fig. IV, A–D. The corresponding cells in the B quadrant have not been seen. Doubtless $2b^{2.2}$ divides, as do $2a^{2.2}$ and $2c^{2.2}$, though at a much later period, but the products of such a division are not to be recognized in fig. 53. In the embryo represented in the figure the trochoblasts can plainly be discerned dorsal to $2a^{2.2.1}$ and $2c^{2.2.1}$; on the ventral side of the embryo they form an irregular cell row. Below this there is a group of small cells which presents to the eye no definite arrangement. These represent the descendants of $2b^{2.2}$; 3a and 3b. Posterior to the blastopore are those cells which in annelids form the ventral plate. These cells barely cover the posterior face of the primary mesoblasts. Just posterior to $2a^{2.2.2}$ and $2c^{2.2.2}$ are one or two small cells on each side whose lineage has not been determined, but which are probably descendants of 3d and 3c. Turning to fig. 55, the relations of the cells surrounding the blastopore are much less clear. On each side of the anterior portion of the blastopore are two cells slightly larger than those surrounding them. These are probably the descendants of $2a^{2.2.1}$, $2a^{2.2.2}$, $2c^{2.2.1}$ and $2c^{2.2.2}$. The blastopore has meanwhile narrowed to an irregular cleft, the cells anterior to it forming a seam, while the cells of the ventral plate have only advanced sufficiently to cover in one of the mesoblasts and part of the other. All of the cells which constitute the rim of the blastopore are small and extremely thin and transparent, making their outlines very difficult to discern, and the exact lineage of any of these impossible to determine. From a comparison of figs. 53 and 55 it is seen that the cells of the ectoderm have crowded toward the vegetal pole from all directions, but especially from the sides. It is also evident that the anterior portion of the margin of the blastopore, between $2a^{2.2.1}$ and $2c^{2.2.1}$, is composed of the descendants of $2b^{2.2}$, 3b and 3c; that the lateral margin is at least partly composed of the descendants of 2a and 2c, unless the small cells which form the blastopore rim in fig. 55 have all slipped in around $2a^{2.2.1}$, $2a^{2.2.2}$, $2c^{2.2.1}$ and $2c^{2.2.2}$. It seems probable that this is not the case, but that the small cells comprising the lateral margin of the blastopore are derived from the second quartette. It is, however, fairly certain that some small cells have pushed in between the descendants of 2a and 2c and the cells of the X group. These must be the descendants of 3c on one side and 3d on the other.

In *Nereis*, Wilson (1892) described these cells $2a^2$, $2c^2$ and $2b^2$ as "stomatoblasts," since they converge to form an arc of cells as the

blastopore closes, and are concerned in the formation of the stomodæum. Mead (1897) states that in *Amphitrite* $2a^2$, $2b^2$ and $2c^2$ come to occupy positions similar to the "stomatoblasts" of *Nereis*, but is in doubt as to their precise fate. In *Capitella*, Eisig (1898) finds that the margin of the blastopore is composed entirely of the products of the second quartette ("œsophagoblasts"). In *Podarke* (Treadwell, 1901) at least one cell from the second quartette forms a portion of the stomodæal wall, as do also products of 3a, 3b and 3c. In *Arenicola* (Child, 1900) eight products from the third quartette function as stomatoblasts and form an arc of cells similar to that formed by the stomatoblasts of *Nereis*.

Various conditions are found among the Mollusca. In *Ischnochiton* (Heath, 1899) products of both the second and the third quartettes take part in the formation of the blastopore lips; this appears to be also the case in *Planorbis* (Holmes, 1901), while in *Trochus* (Robert, 1903) the lips are formed at first by cells derived from both the second and third quartettes, but later the products of the second quartette are excluded from the rim of the blastopore.

In conclusion, it may be said that in *Dinophilus* no one set of cells can be denominated stomatoblasts, but that products from both the second and third quartettes take part in the formation of the blastopore rim. The products of $2b^{2.2}$, 3a and 3b, since they lie immediately in front of the blastopore, probably contribute to the formation of the stomodæum. The fate of the cells $2a^{2.2.1}$, $2a^{2.2.2}$, $2c^{2.2.1}$ and $2c^{2.2.2}$ is problematical. It is possible that they, too, contribute to the formation of the stomodæal wall. On the other hand, it seems pretty certain that but a small part of 3c and 3d contribute to the formation of the stomodæum. Their position, just anterior to the cells of the X group, which is not materially altered during the shifting of the ectoderm, together with their large size, makes it possible that they contribute largely to the lateral ectoderm of the head.

Mesoblast of ectodermal origin (larval mesoblast, Lillie; pædomesoblast, Eisig) has been described for a number of Mollusca, and the annelids *Podarke* (Treadwell, 1901), *Capitella* (Eisig, 1898), *Aricia* (Wilson, 1898) and *Thalassema* (Torrey, 1903). Schimkewitsch (1895) describes cells from the ectoderm in the anterior part of the *Dinophilus* embryo as migrating into the body cavity and there contributing to the mesenchyme. Certain cells, of whose exact lineage I am ignorant, belonging to the first quartette, do invaginate (figs. 57 and 58), and it is quite possible that one or more of them may give rise to the mesenchyme of the head and the mouth segment region; but it is my belief

that ectomesoblast, if such exists, is very small in amount, and that the greater part, if not all, of the mesenchyme of the adult as well as the ovaries are formed from 4d.

VIII.—History of the Fourth and Fifth Quartettes.

(1) *The Entomeres.*

The entoderm, at the time 4d is formed (fig. 17), consists of 2A, 2B, 1C and 3D. The origin of the third quartette and the primary mesoblast cell 4d have already been described. The other three members of the fourth quartette arise by an equal and leiotropic cleavage. Figs. 23 and 24 show the origin of 4c, fig. 29 that of 4a, while figs. 33 and 41 show 4b already formed. At the 72-cell stage 4D has divided dexiotropically into 5D and 5d (fig. 33), the latter product being the smaller. The inequality of this division compensates, so to speak, for the inequality of the division preceding, since at the completion of the seventh cleavage the original macromeres 5A, 5B, 5C and 5D are alike in size. After the division of 4D, 4C, 4A and 4B divide equally and dexiotropically to form the fifth quartette (figs. 51 and 52). The original macromeres now form a cross, the arms of which lie radially. Since up to the last division the macromeres lay interradially, it is evident that a rotation of the macromeres through an arc of 45 degrees has been brought about. This rotation has taken place in an anticlockwise direction, viewing the ovum from the animal pole, as can be seen by a comparison of figs. 50 and 52. This movement is due to the reduction in size of the macromeres and their superficial position at the seventh cleavage. During this cleavage the members of the fifth quartette are, on the other hand, held in place by their greater surface contact with the surrounding cells of the egg, so that the macromeres 5A, etc., are rotated, as were the members of the first quartette at the time of their origin. A similar rotation apparently occurs also in *Amphitrite, Clymenella* (Mead, 1897) and *Arenicola* (Child, 1900).

After the division of the fourth quartette the entomeres cease dividing and enter a resting stage which continues until after the closure of the blastopore. They now form a thick, roughly ovoid mass of cells, fourteen in number. At the centre of this mass on the ventral side (fig. 52) lie the four macromeres, 5D posterior, 5B anterior, while 5C and 5A lie laterally. Alternating with the macromeres are the four members of the fifth quartette, while at the outer ends of the cross formed by the macromeres lie the three pairs of entomeres belonging to the fourth quartette. This relation is very similar to that described

by Wilson (1892) for *Aricia*. In both cases this arrangement is probably brought about by the mechanical processes involved in spiral cleavage. Each cell approximates in shape a four-sided prism, the nuclei lying in the lower ends of the cells, near their ventral surface. As the blastopore narrows, however, these nuclei begin to move inward (figs. 54 and 56). This movement is associated with a change in shape of the entomeres, which in turn is part of the process of gastrulation. As the blastopore lips draw together (figs. 53 and 55) the lower or vegetal pole ends of the entomeres grow smaller, while the upper or animal pole ends become larger (figs. 54 and 56). These cells thus change from a prismatic to a pyramidal shape. Viewed in a sagittal optical section their outlines radiate fanwise from the blastopore (fig. 56). This condition is figured by Repiachoff (1886) and also by Schimkewitsch (1895). This peculiar phenomenon may be a reminiscence of a time when the type of gastrulation was embolic and not epibolic, and the latter condition may have been brought about by acquisition of food yolk during the phylogeny. Such acquisition of yolk is a secondary character commonly associated with a change from larval to direct development, and it is perfectly possible that a change from emboly to epiboly may have been brought about in this manner. The nuclei have meanwhile undergone changes in structure as well as in position. The chromatin, which before was distributed in the nuclear vesicles in the form of small granules, is now concentrated in each vesicle in one deep staining chromatin nucleolus (fig. 56). This condition is not an uncommon one in resting cells, and is also seen in the entomeres of *Crepidula* (Conklin, 1897, figs. 52, 53 and 54).

The further history of the entoderm I have not been able to follow in detail. At a period when the stomodæum has assumed the position of the definitive mouth, the entoderm cells are seen to have multiplied somewhat and to have assumed an arrangement quite different from that found in earlier stages. In fig. 57, which is a horizontal, optical section of a stage when the stomodæum is just making its appearance, the entoderm cells have still the radial arrangement which they assumed at the time of the closure of the blastopore. In fig. 59, a sagittal section of a much later stage, the arrangement is totally different; the entoderm cells have multiplied and are now arranged in a more or less definite layer about a small central lumen.

(2) *The Mesomeres.*

At the 29-cell stage 4d is but just formed and lies on the lower side of the cleaving ovum, below and in front of X and to the left of the

mid-line. Very soon after its formation 4d divides again. This division parts it, by a bilateral cleavage, into two equal cells, the primary mesomeres M and M. This division is illustrated in figs. 28 and 44. The primary mesomeres remain undivided up to the 72-cell stage, when they undergo a division of great interest. This division is shown in figs. 33 and 36. By it two small cells are budded off anteriorly toward the vegetal pole, and close to the line of juncture of the two mesomeres. This division is, however, not bilaterally symmetrical, *but is, on the other hand, plainly dexiotropic, and follows the law of alternating cleavages.* The product of the left mesomere thus lies in the furrow formed by the juncture of the two mesomeres and the entomeres 5D and 5d. The product of the right mesomeres, on the other hand, lies between its parent cell and 5D. The next division of the mesomeres (fig. 50) is also not symmetrical. By this division the left mesomere buds off a small cell on its left anterior surface, that is, leiotropically; on the other hand, the right mesomere violates the law of spiral cleavage by dividing in the same direction as before and placing a small cell to the left of its first product (fig. 50). The next division marks the beginning of true teloblastic cleavages. Each mesomere in this division buds off dorsally and laterally a small cell. Just how many of these divisions occur before the closure of the blastopore I cannot say, but probably not more than two. After the closure of the blastopore the mesomeres begin to shift apart, moving laterally, forward and somewhat dorsally. In the horizontal optical section shown in fig. 57 they have reached a position which, I think, is their final one. In this movement they not only have changed their position with regard to one another, but also with regard to the median plane of the embryo. Up to the time of the closure of the blastopore the mesomeres are situated at the left of the ventral mid-line, but, as shown in fig. 57, at the close of this movement the mesomeres are bilaterally situated with respect to the median plane of the embryo.

The cause of this shifting apart is not clear, but I think it is to be explained by the peculiar shape of the entomeres. These are, at the time the blastopore closes, pyramidal in form, with their apices at the blastopore. This point, as will be seen by reference to figs. 53 and 55, is just anterior to the junction of the mesomeres. After the ectoderm of the ventral plate has enclosed the mesomeres, they are subjected to a pressure from the ectoderm which tends to force them inward against the narrow ends of the entomeres. These latter press in between the mesomeres and wedge them apart. They are, however, prevented from passing backwards, and in fact compelled to move

forward, by contact with the cells of the X group. In *Thalassema* there occurs a similar shifting apart of the mesomeres, which Torrey (1903) also explains as being caused by pressure of the entomeres.

The position occupied by the mesomeres is at first sight somewhat different from that occupied by similar cells among the mollusks and annelids. In the majority of these forms the mesomeres, soon after their formation, are invaginated into the cleavage cavity. In *Dinophilus* they remain on the exterior until covered by ectoderm, when they move laterally to the entoderm. There was, however, at an early stage a cleavage cavity between the ectoderm and the entoderm, and had the mesomeres moved into it, then their behavior would have been that of the corresponding cells in most mollusks and annelids. As it is, their migration into the cleavage cavity is postponed until a later period of the history of the embryo, but their final position is not essentially different from that of other forms.

In fig. 57 a band of mesoblast cells is seen on each side of the entoderm, running forward from the mesomeres, which are in division. The mesoblast is also shown in fig. 59 ventral to the entoderm.

The later history of the mesoblast has not been followed out in detail. It is my hope to be able at a later period to determine precisely to what organs and tissues the mesomeres contribute, but it seems fairly certain that they give rise to the mesenchyme and sex organs (ovaries) of the adult.

Of especial interest would be the fate of the first two products of each mesomere, in the light of the discovery made by Conklin (1897) that part of 4d is in *Crepidula* entodermal.

Following Conklin's discovery, Wilson (1897) in *Nereis*, Treadwell (1901) in *Podarke* and Torrey (1903) in *Thalassema* found that part of the mesomeres in these forms was entodermal. I have not been able to follow out the fate of the first two products of each mesomere, since their small size and position causes them to become inextricably blended with the ectodermal cells surrounding the posterior lip of the blastopore. The position of three of these cells in the mid-line suggests, however, that their fate may be different from that of the other products of the mesomeres.

IX.—Axial Relations.

The discussion of the axial relations of the *Dinophilus* embryo falls naturally under two heads, viz.: (1) The relation of the first and second cleavage planes to the future median plane of the adult, and (2) the shifting of areas which occurs in relation to the closure of the blasto-

pore. Both of these questions have received much attention from embryologists, and have been so thoroughly discussed by the writers on cell-lineage that it would be superfluous for me to attempt here to treat the subject at length, and so I shall confine myself to stating what these relations are in the *Dinophilus* embryo and to comparing them with a few other forms.

As already mentioned, the cell 2d is of very great value as a landmark. From its origin up to the time when the last traces of it are seen, it marks the posterior end of the embryo. Of course it may be said that since the first three cleavages are of the spiral type, and not of the bilateral type, and since x^1 and x^2 are not exactly equal in size, that the centre of 2d before these cleavages could not well be situated at the same point as that of 2d after them. This, however, is not a point of practical importance. To all intents and purposes the centre of the cell 2d lies in the future median plane of the embryo. The animal and vegetal poles lie in this plane, so that its relation to the cleaving egg can now be determined.

2d, after its formation, lies in the furrow between 1c and 1D. A glance at the figures from fig. 10 to fig. 33 shows that the second cleavage plane between the macromeres, up to the 72-cell stage (fig. 33), very nearly coincides with the median plane of the embryo, since both 2d and the vegetal pole lie in this furrow. At the 72-cell stage, however, these relations are beginning to undergo a change. As described under the history of the entomeres, the macromeres undergo a rotation through 45 degrees, bringing 5D and 5B into the former plane of the second cleavage. This point will be made clear by reference to figs. 50 and 52. The median plane of the embryo and adult then passes through 5B and 5D, and forms an angle of 45 degrees with the plane of the second cleavage between the original macromeres. In conclusion, it follows, since 2d marks the posterior region of the embryo, and since during the early cleavage stages it lies in the furrow between the posterior macromeres, that the second cleavage plane does coincide in a general way with the future sagittal plane of the embryo, although it is evident that at the 4-cell stage the cleavage plane between C and D must pass to the right of the sagittal plane of the embryo. This result is at variance with the results obtained among the Annelida by most writers, though Wilson (1892) found that the second cleavage coincides with the sagittal plane of the future embryo. Much importance, however, cannot be attached to these relations of the entomeres with the embryo, since they are the result of shiftings between the macromeres and micromeres, which may occur at an early period. In

Dinophilus, for example, the members of the first quartette when first formed lie in the furrows between the macromeres; at the next cleavage they are shifted in a sinistral direction, so that they lie precisely over their parent macromeres (cf. figs. 8 and 10), and this relative position is retained up to the formation of the fifth quartette. In *Amphitrite* (Mead, 1897) and *Arenicola* (Child, 1900) this sinistral rotation does not bring the micromeres so precisely over the corresponding macromeres.

In considering the relation of the cleavage plane with the embryonic axis, the micromeres are of far more importance than the macromeres. An examination of the figures of the later cleavages will show that the planes of the first and second cleavages as traced over the entire embryo do not coincide with its transverse and sagittal planes, but that here the rule laid down by Lillie (1895) holds good, viz.: "The members of the odd generations of ectomeres, as well as the entomeres, are distributed two each, right and left of the middle line; those of the even generations are placed anterior, posterior, right and left."

The shifting of the axis, which is largely concerned in bringing about the closure of the blastopore, has been already described in chapter V. It was there spoken of as a forward movement of the first quartette, or rather of the ectoderm of the anterior half of the embryo. This shifting, I think, might better be looked upon as a shifting backward of the ectoderm of the posterior half of the embryo, considering the animal pole as the fixed point. In the first case the blastopore would be closed from in front backward; in the latter from behind forward, which brings it into line with what we know of other forms. In either case the egg axis is bent through an angle of nearly 90 degrees, but not quite, since the animal pole is, as Wilson (1892) showed to be the case in *Nereis*, probably slightly dorsal to the anterior pole of the embryo. This shifting of areas during the process of gastrulation is almost precisely similar to that which occurs in *Crepidula*, as described by Conklin (1897). There is in that form a bending of the egg axis through 90 degrees, and also caused by rapid growth of the cells of the D quadrant, which pushes forward the ectoderm of the aboral surface over the entoderm, the vegetative pole being apparently the fixed point. This condition is attributed by Conklin to the accumulation of yolk in the entoderm cells, and doubtless this is the cause of the similar relations existing between entoderm and ectoderm in *Dinophilus*, the solid yolk-laden mass of entoderm cells altering their form and relative positions but little, while the ectoderm is shifted over them.

X.—The Phylogenetic Relationships of Dinophilus in the Light of Its Early Development.

In this chapter I wish to briefly compare the early development of *Dinophilus* with that of other groups, and to consider what light a study of its cell-lineage sheds on its systematic position. That the study of cell-lineage is of value in determining relationship has been abundantly shown by the results accomplished in this particular branch of zoological research. These have in a most striking degree corroborated the results attained by the study of comparative anatomy, and have further shown that in a large number of forms, representative of large and important groups, the characters of the various forms as manifested in the cleavage are as constant as the anatomical characters, and must therefore be as truly inherited. Furthermore, since cœnogenetic changes may be supposed to affect the later stage of development first, we may expect to find the earlier stages retaining longer their primitive characters, although even the earliest stages have been affected by precocious segregation and are no longer highly primitive. The study of the early development cannot be regarded as a sure or certain guide in determining relationship in every case, yet it may, I think, be very properly called to aid in the determination of the relationships of doubtful forms. In the case of *Dinophilus*, the cleavage has presented such startling and accurate resemblances to the chætopod annelids that it seems impossible that they do not indicate relationship, for so many and minute correspondences could hardly have arisen independently. I have already stated these resemblances separately, but wish here to bring them together, in order that their force may be more apparent.

In the first place the manner of origin of the germ layers—ectoderm arising from the first three quartettes, mesoderm from the left posterior member of the fourth quartette, and entoderm from the remaining cells—brings *Dinophilus* into a list of forms already large and still increasing, containing members of the Lamellibranchia, Gasteropoda, Polychæta and Echiuridæ. In common with many Annelida and Mollusca the larger part (or all) of the ectoderm of the trunk is derived from one cell, the posterior member of the second quartette. A little later the resemblances to the annelids become more marked. In the development of the second preoral ciliated band of *Dinophilus* is recognized the prototroch of the trochophore, and the earliest rudiment of the brain appears at the same point as the annelid "Scheitelplatte." There are, however, in the cleavages themselves resemblances to the cleavage of the polychæte annelids which are most

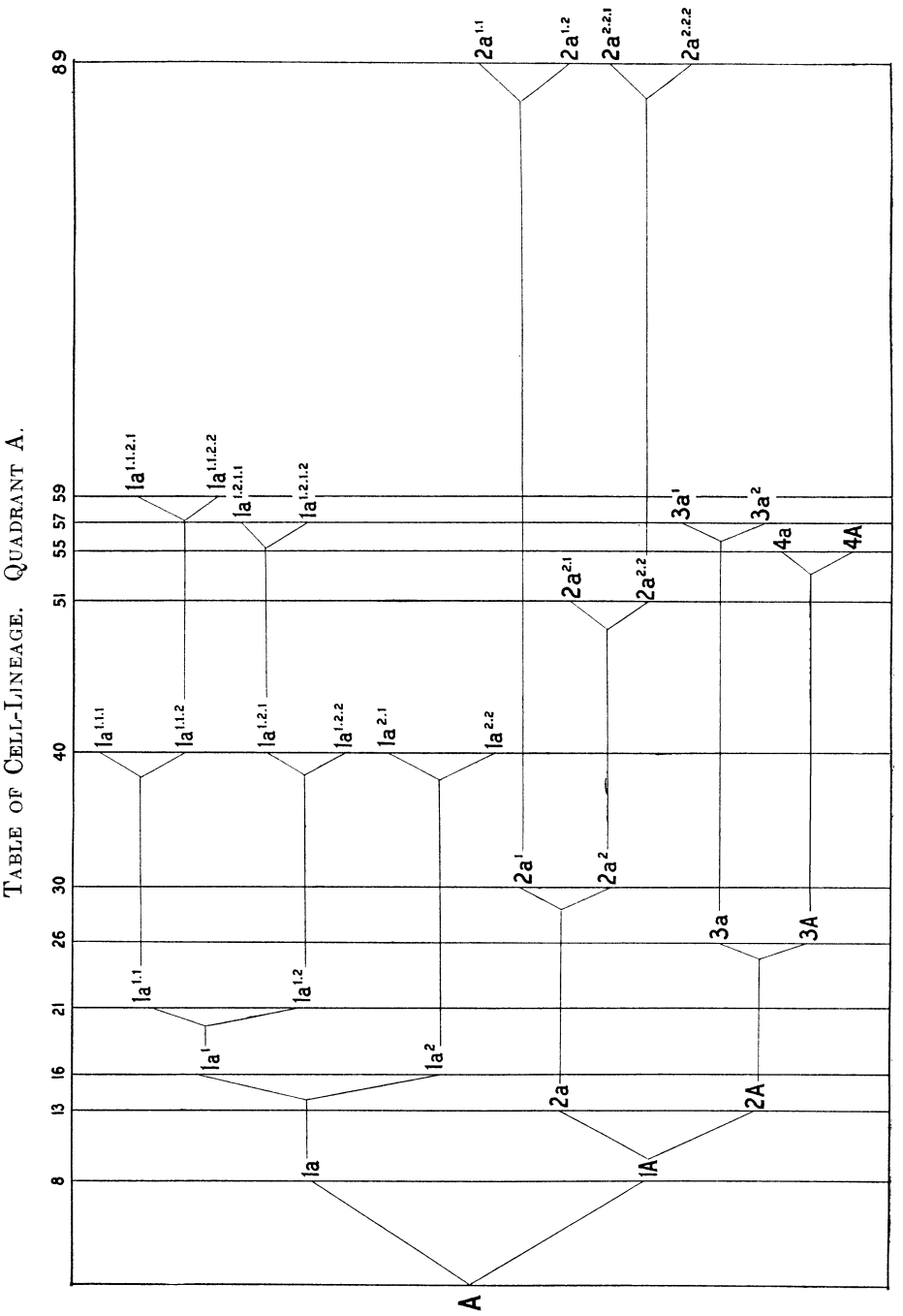
striking, viz., in the origin of the bilateral cleavages. In the cross and in the products of 2d the transition from the spiral type of cleavage to the more specialized bilateral type occurs in precisely the same cells and in precisely the same directions as in the Polychæta. Moreover, the second bilateral divisions of the cells of the posterior arms of the cross continue this resemblance. All these characters, if such they may be called, when viewed as a whole, point in no uncertain way to a descent from the annelid stem, and at a point not far from that at which the Polychæta arose.

If we consider, as I think at present we must, that the trochophore is a larval form common at least to the Annelida, we cannot regard the development of *Dinophilus* as primitive. This view is upheld by many features of the cleavage, especially by the many departures from the spiral type exemplified in the divisions of the primary trochoblasts and many other cells, and also in the discrepancy between the size of 2b and 3b as compared with their sister cells. Further evidence of secondary change is found in the enormous size of 2d and 4d, which have acquired a very large amount of cytoplasm in order to supply material to build up the trunk region, which in the trochophore is ordinarily acquired from the exterior through active feeding. Besides these there is the retarded development of the adult organs, which do not become functional until the animal is nearly ready to hatch, but which must have been primitively functional at a much earlier period. Such organs are the intestine and prototroch. The latter, together with the rosette, and possibly the perianal band of cilia, which may represent the paratroch, are the sole relics of organs peculiar to the trochophore. It is to be regarded as doubtful if even these would now be recognizable, were it not for the fact that these are also concerned in the formation of adult organs. How quickly such a larval organ as the prototroch may disappear is illustrated in the development of *Sternaspis* (Child, 1900), where the prototroch has entirely disappeared, though this larval organ is highly developed in related forms. Thus in the light of the cleavage, as well as in that afforded by the more recent work on the anatomy of *Dinophilus*, Metschnikoff's conjecture appears almost prophetic. *Dinophilus* probably is to be regarded as a "stationäre Annelidenlarva," but one in which the larval stage has become an end stage toward which the development tends and which has become correspondingly modified.

Postscript.

Since the above paper was presented for publication there have come to hand two papers by Prof. John H. Gerould: "The Development of Phascolosoma" (*Arch. Zool. exp. et gen. II, 2, 1904*) and "Studies on the Embryology of the Sipunculidæ: I. The Embryonal Envelope and Its Homologies" (*Mark Anniversary Volume, Art. XXII*). In the former of these two papers Prof Gerould shows that in *Phascolosoma* the ectoderm arises from the first three quartettes of micromeres, the mesoderm from 4d, and the entoderm from the remaining cells, thus adding a representative of the Sipunculidæ to the list of forms mentioned in section IV (4) of the present paper. The author further points out the presence of a typical annelid "cross" and "rosette" in *Phascolosoma*, that the prototroch of the larva is formed from the sixteen "primary trochoblasts," and that the "somatic plate" arises from 2d. The second paper mentioned, among other interesting facts concerning the relation of the larva of *Sipunculus* and *Phascolosoma*, establishes the homology of the "serosa" of *Phascolosoma* with the prototroch of *Sipunculus*.

TABLE OF CELL-LINEAGE. QUADRANT A.



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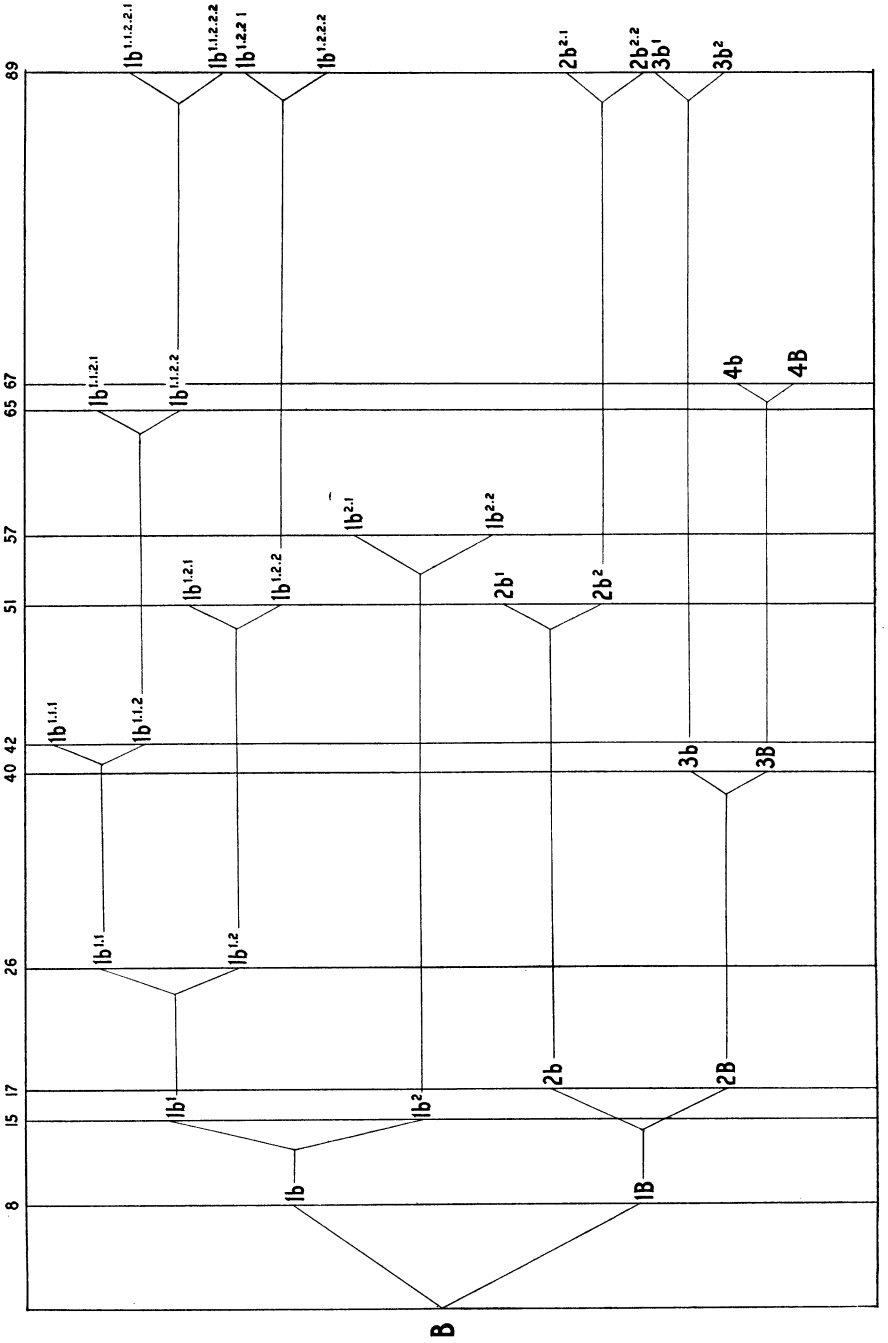

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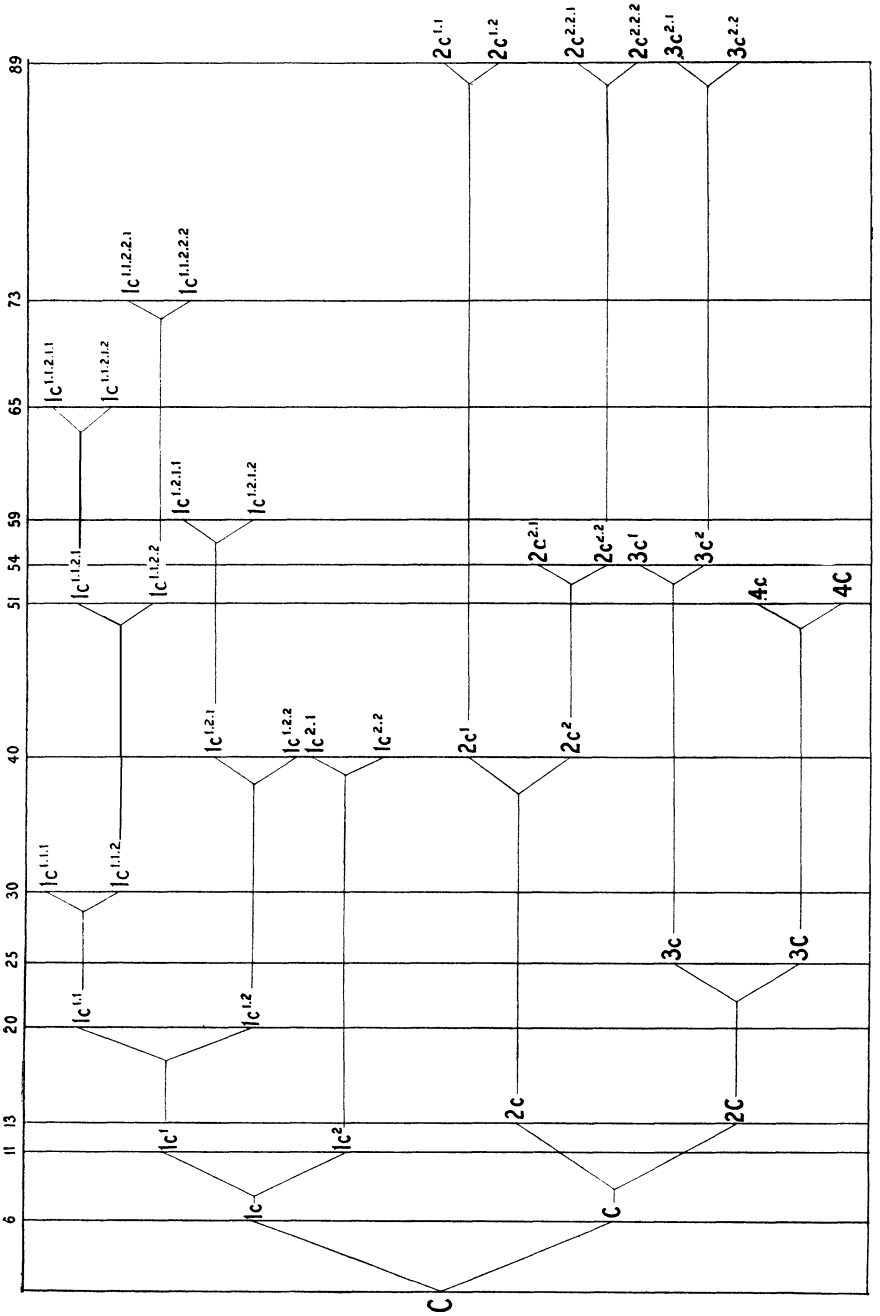

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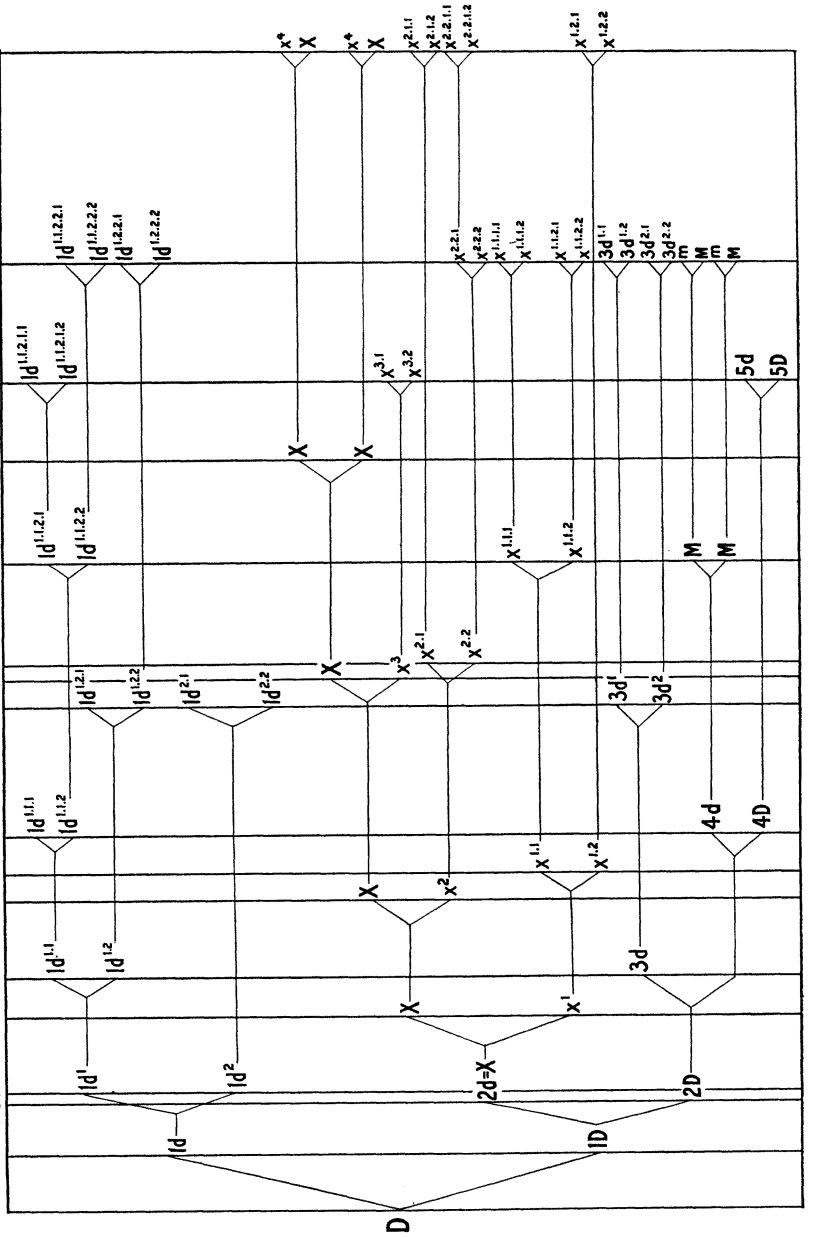

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EXPLANATION OF PLATES XLIII–XLVIII.

The figures have, with few exceptions, been drawn with the camera lucida at the table level under Zeiss homo. imm. $\frac{1}{12}$, oc. 2. With whatever lenses used, however, all the figures were drawn to the same scale of magnification.

REFERENCE LETTERS.

bl., Blastopore.
br., Brain.
br. com., Commissure of brain.
gl., Gland
int.l., Lumen of intestine.
m., Mouth.
mes., Mesoderm.
pr., Proctodæum.
pro., Proboscis.
st., Stomodæum.
sto.l., Lumen of stomach.
v.p., Ectoderm of ventral plate.

PLATE XLIII, Fig. 1.—Unsegmented ovum, after the expulsion of the second polar body. The female pronucleus lies at the animal pole of the egg, surrounded on its lower side by a large aster. The sperm nucleus, with its accompanying aster, is seen to the right of and below the centre of the ovum.

Fig. 2.—Unsegmented ovum after union of the two pronuclei.

Fig. 3.—Early anaphase of first cleavage spindle. The cell-body is beginning to elongate.

Fig. 4.—Two cells. Prophases of spindles for second cleavage.

Fig. 5.—Two cells. A–B in metaphase, C–D in anaphase of division.

Figs. 6 and 7.—Four cells. Spindle fibres still connecting A and B. Animal pole.

Fig. 8.—Seven to eight cells. Animal pole. 1c and 1d formed and in pro-

phase of next division. 1a just formed and 1b in process of formation. 1c is taking in one of the polar bodies.

Fig. 9.—Nine cells, left side. 2d just formed; 1d preparing to give off $1d^2$.

Fig. 10.—Twelve cells, animal pole. $1a^2$ just formed.

Fig. 11.—Thirteen to fourteen cells, animal pole. 1b about to divide.

Fig. 12.—Thirteen to fourteen cells, right side. 2c just formed; 2d=X dividing to form x^1.

PLATE XLIV, Fig. 13.—Thirteen to fourteen cells, left side. 2a in process of formation.

Fig. 14.—Twenty-six cells, animal pole. $1b^{1.2}$ just formed; $1c^{1.1}$ and $1d^{1.1}$ preparing to form rosette.

Fig. 15.—Twenty-six cells from right side. Spindle fibres connecting 3C and 3c; $1c^2$ preparing to divide.

Fig. 16.—Twenty-six cells, left side. 2a and 3D preparing to divide; spindle fibres connecting X and x^2, and 3A and 3a.

Fig. 17.—Twenty-six cells, vegetal pole. $x^{1.2}$ forming; 3D in metaphase of division.

Fig. 18.—Twenty-eight cells, left side. $2a^1$ formed; cell body of 3D elongated.

Fig. 19.—Twenty-eight cells, right side. 2B in division.

Fig. 20.—Forty cells, animal pole. $1c^{1.1.2}$ and $1d^{1.1.2}$ preparing for their first bilateral division.

Fig. 21.—Forty cells, right side.

PLATE XLV, Fig. 22.—Forty cells, left side.

Fig. 23.—Forty-two cells, right side. 4c just formed.

Fig. 24.—Forty-two cells, vegetal pole. 4d preparing for bilateral division.

Fig. 25.—Fifty-four cells, animal pole. Bilateral division of the posterior stem cells of the first quartette completed.

Fig. 26.—Fifty-four cells, right side. $2c^{2.1}$ formed.

Fig. 27.—Fifty-four cells, left side.

Fig. 28.—Fifty-four cells, vegetal pole. Bilateral division of 4d completed; X preparing for bilateral division.

Fig. 29.—Fifty-three cells, anterior end. Divisions of 3A, 2b, 3b, 1b and $2c^2$ shown.

Fig. 30.—Seventy-two cells. Division of cells of posterior arms of cross; arm of cross in the B quadrant just formed.

PLATE XLVI, Fig. 31.—Seventy-two cells, right side.

Fig. 32.—Seventy-two cells, left side.

Fig. 33.—Seventy-two cells, vegetal pole. $x^{2.2}$ divided; 5d just formed.

Fig. 34.—Eighty-one cells, right side. $2c^{2.2}$ and $2c^{1}$ divided.

Fig. 35.—Eighty-one cells, left side. $2a^{2.2}$ and $2a^1$ in process of division.

Fig. 36.—Eighty-one cells, vegetal pole. Dexiotropic division of M. and M.

Fig. 37.—Eighty-nine cells, animal pole. Division of $1b^{1.2.1}$.

Figs. 38 and 39.—Eighty-nine cells, right and left sides respectively.

PLATE XLVII, Fig. 40.—Eighty-nine cells, vegetal pole.

Figs. 42–49.—Products of 2d=X viewed from the posterior end. Fig. 42 was drawn from the same ovum as represented in Fig. 20; Fig. 44 likewise corresponds to Fig. 30; Fig. 45 to Figs. 34–36; and Fig. 47 to Fig. 37.

Fig. 50.—Vegetal pole. Formation of 5a and second division of M and M.

Fig. 51.—Vegetal pole. 4c divided; 4a and 4b dividing.

Fig. 52.—Division of entomeres completed.

PLATE XLVIII, Figs. 53 and 55.—Two stages during the closure of the blastopore. They show also the division of X and X to form x^6 and x^6.

Figs. 54 and 56.—Optical sagittal sections of same, showing change in form of the entoderm cells and the inward migration of their nuclei.

Fig. 57.—Horizontal optical section of embryo at the time of the appearance of the stomodæum. The mesomeres, which are seen in division, have moved apart. In front of each mesomere is seen the band of mesoblast to which it has given rise.

Fig. 58.—Optical sagittal section of same stage, showing formation of

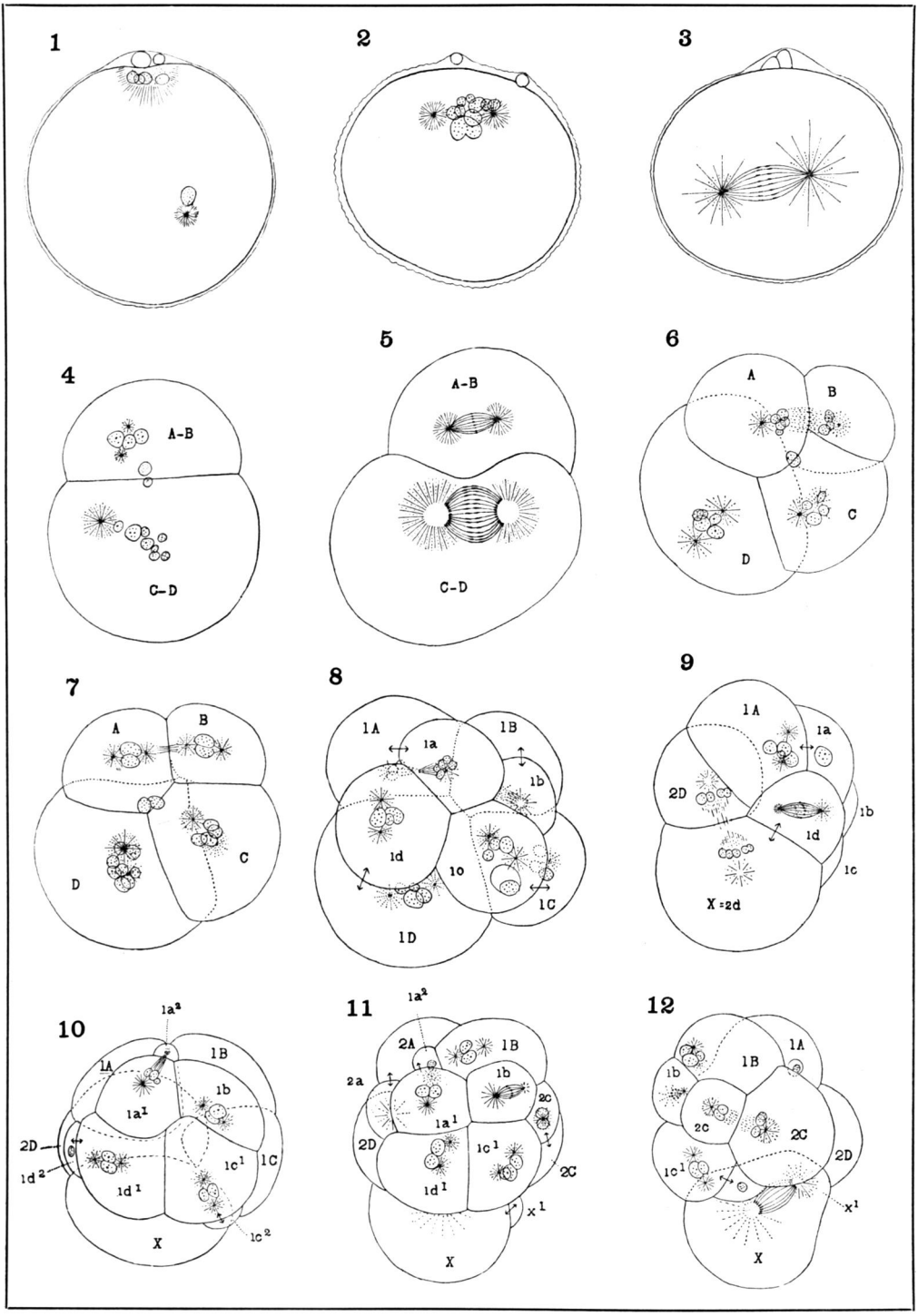
stomodæum. At the anterior end is also seen the ectodermal thickening which gives rise to the brain.

Fig. 59.—Sagittal section of a much later stage than the last. The stomodæum has assumed the position of the definitive mouth, while the entoderm cells have multiplied and arranged themselves about the rudiment of the intestinal lumen. Below the entoderm cells is seen the mesoderm.

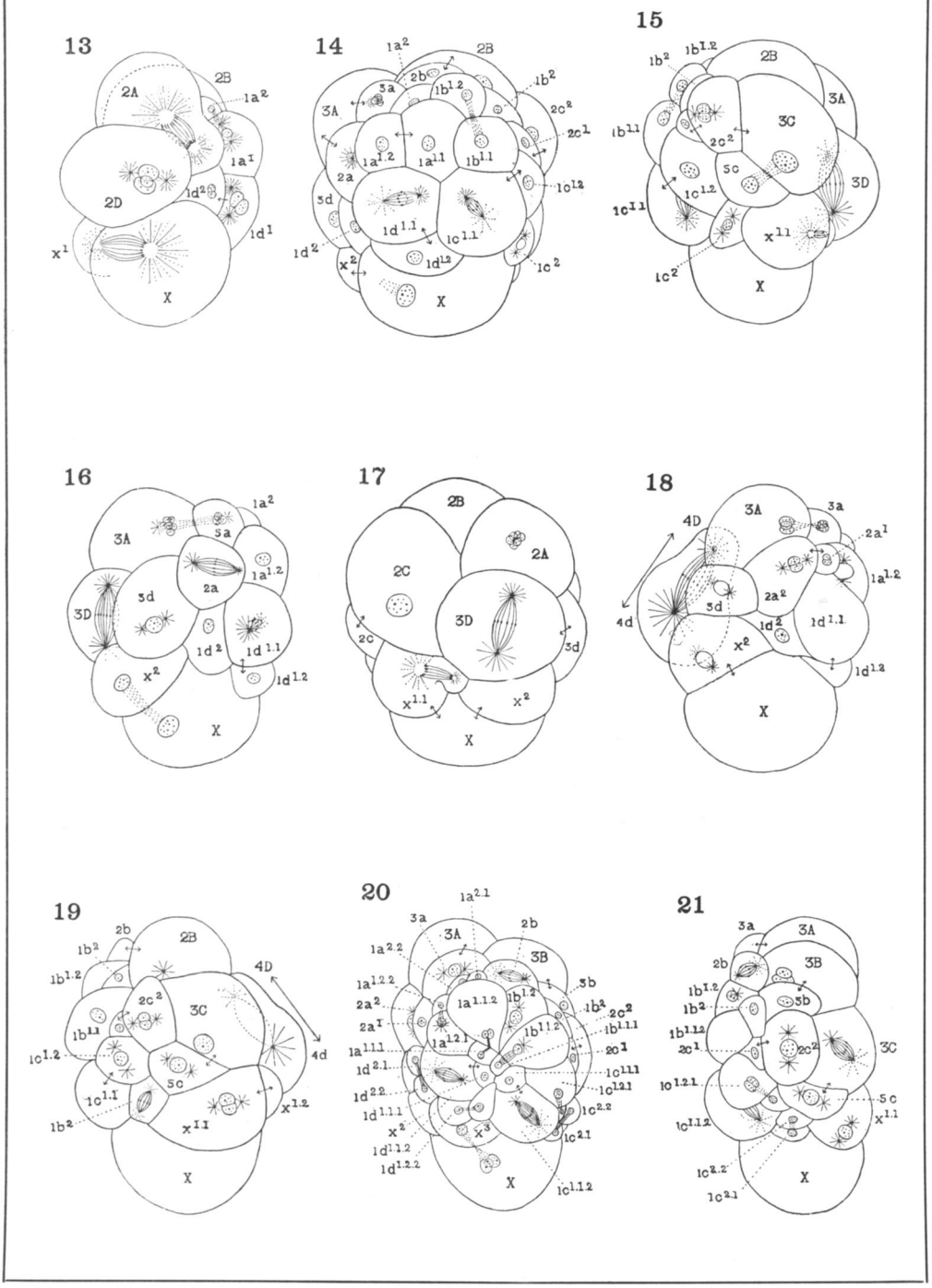
Fig. 60.—Sagittal section of a late stage. The entoderm cells now form an epithelial layer about a well-defined lumen. The rudiment of the intestine is making its appearance.

Fig. 61.—Horizontal section of an embryo with four segments.

Fig. 62.—Transverse section of a stage similar to that illustrated in Fig. 60, showing the relations of the three germ layers.



NELSON ON DINOPHILUS.



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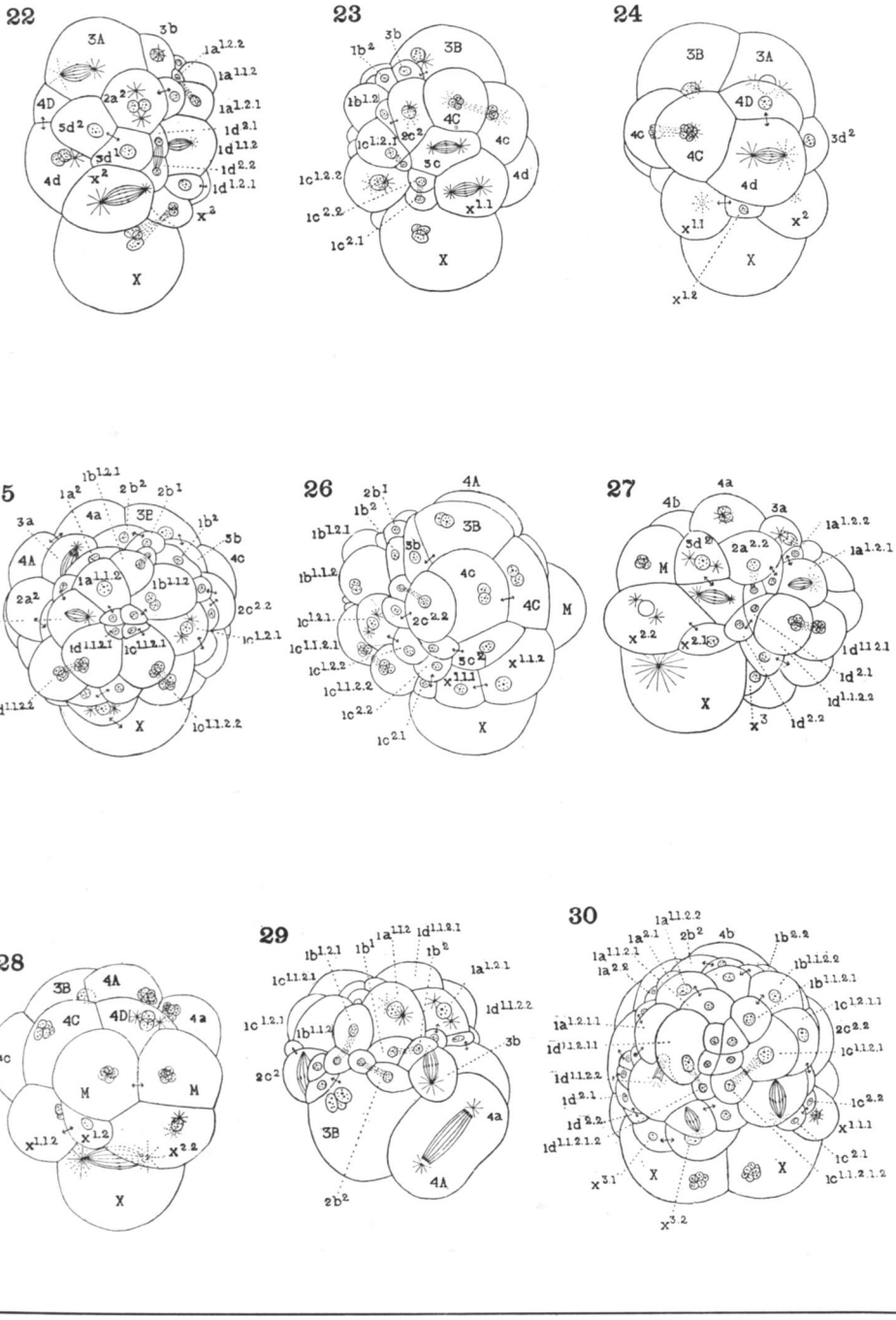

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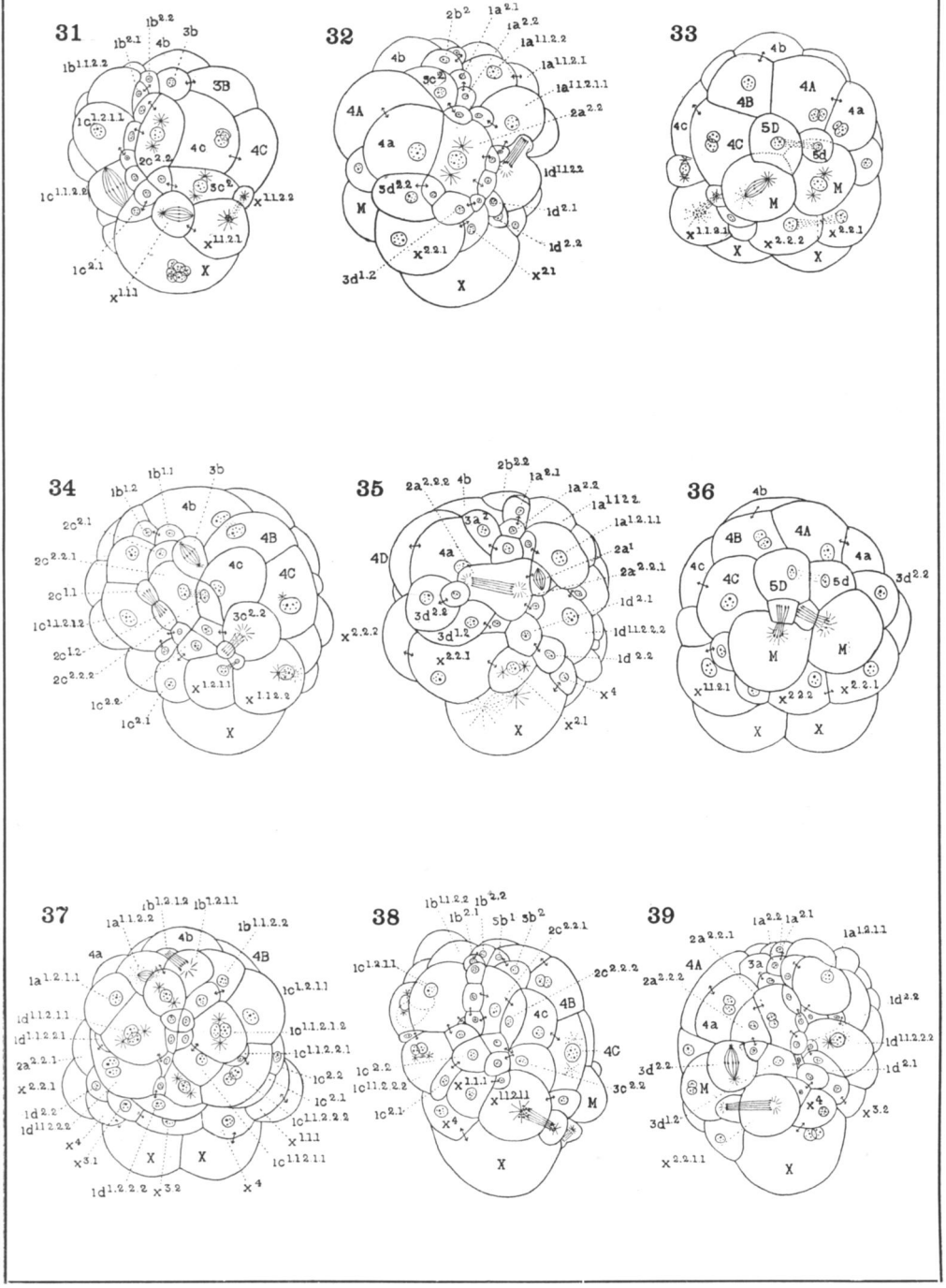

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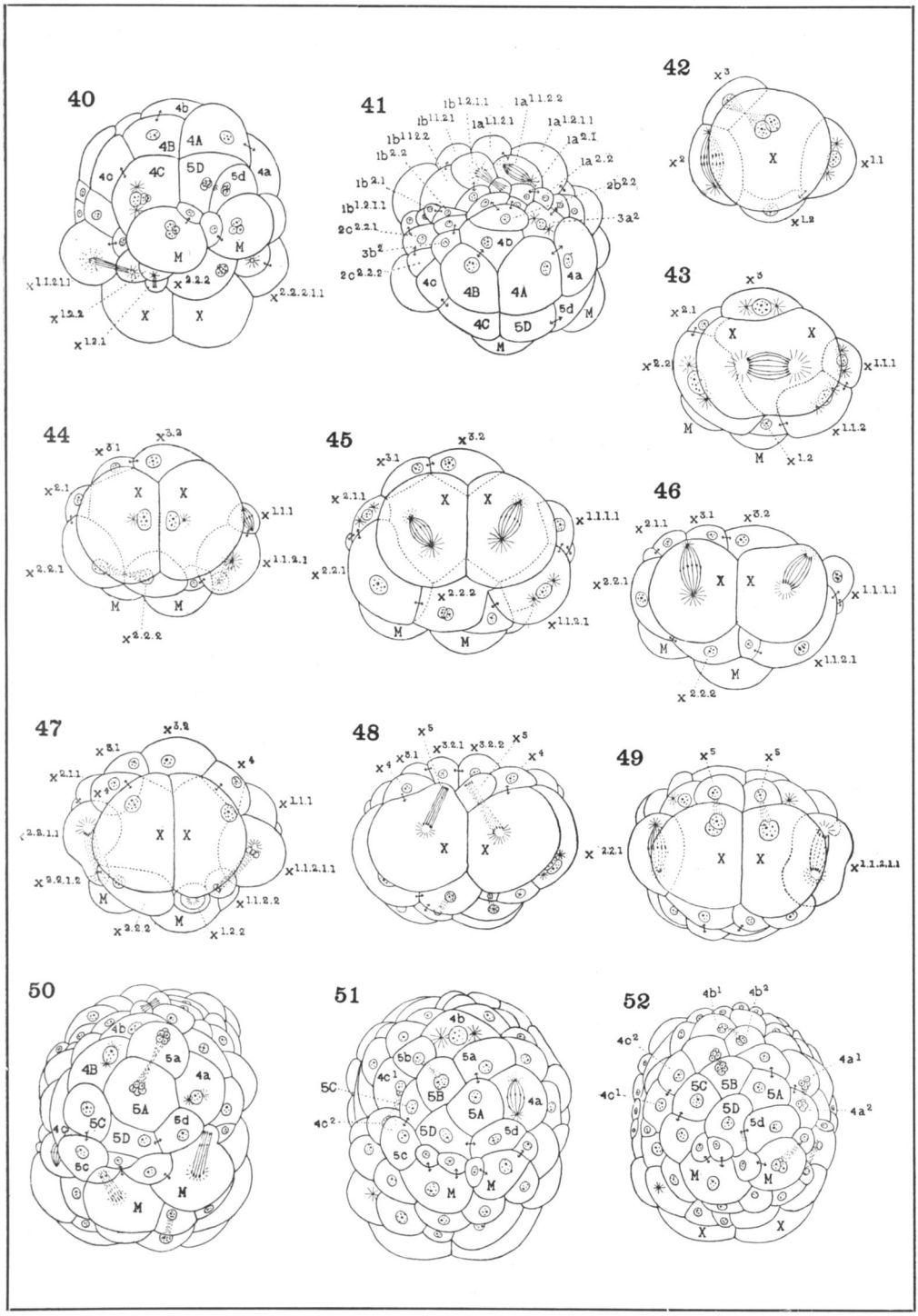

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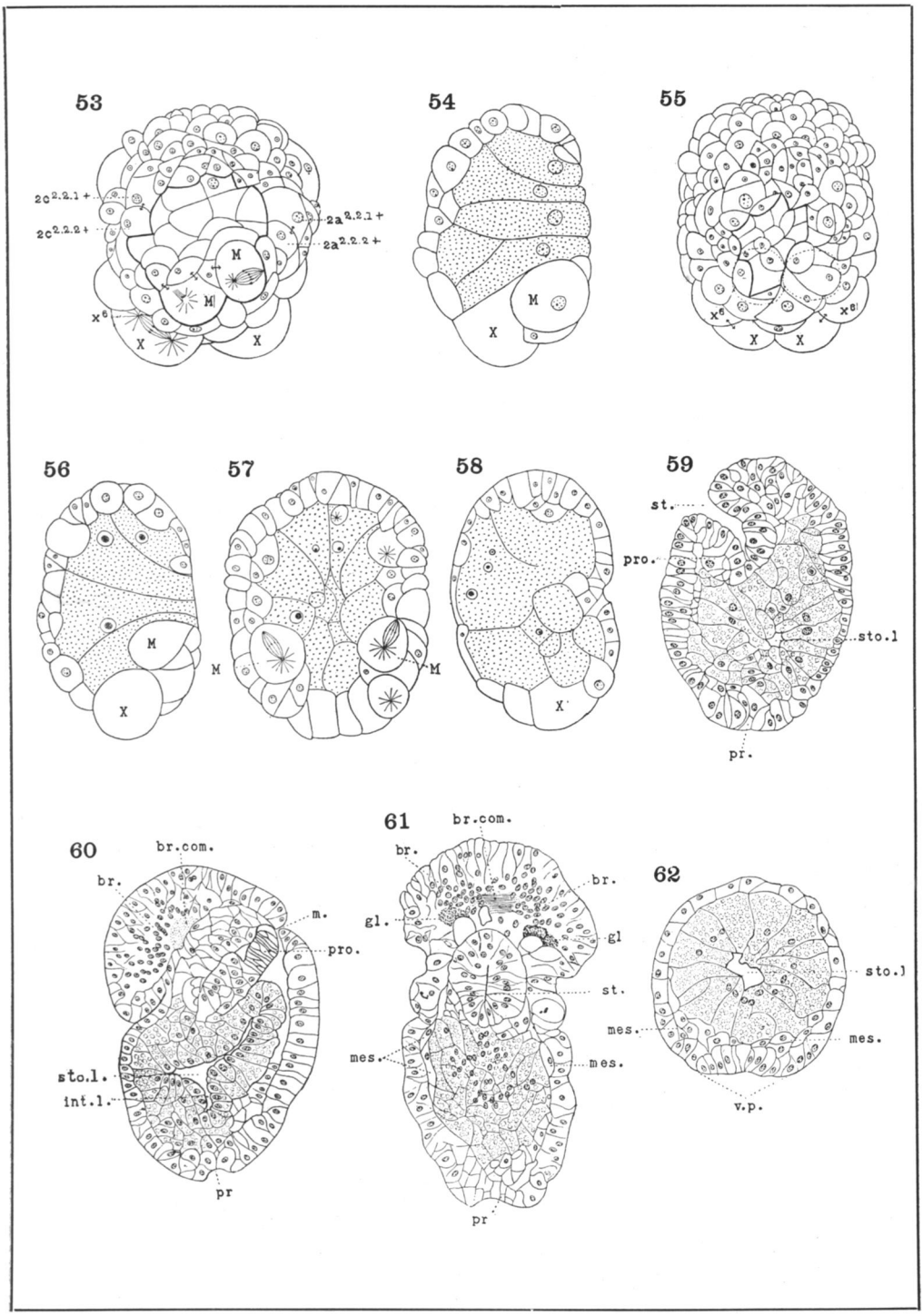

NELSON ON DINOPHILUS.